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Improving the accuracy of Kováts' retention indices in isothermal gas chromatography

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Abstract

Isothermal Kováts' retention indices are currently reported as whole numbers, and are frequently deduced from a linear least mean squares fitting of the logarithms of adjusted retention times of a number of *n*-alkanes versus carbon number, following an iterative method that minimises errors. The currently accepted accuracy is about one retention index unit for apolar stationary phases, and lower for polar stationary phases. This paper presents results that show how the accuracy of the retention index may be safely reported to one-tenth of a retention index unit by the use of a non-linear equation, with present day gas chromatographs without electronic flow controllers. Results are presented that prove the correctness of the retention indices found for several substances on one particular capillary column. Hints on the minimum retention times needed to achieve the 0.1 retention index accuracy are mentioned, for retention times recorded in minutes and in seconds. According to results of this paper, two chromatograms, run under the appropriate conditions, are sufficient to obtain the desired accuracy. The method proposed in this paper does not require knowledge of the hold-up time of the chromatogram. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

One of the most powerful tools in analytical chemistry is without doubt gas chromatography (GC). Unsurpassed as a separation technique, it produces one single value for each analyte which may be used for identification purposes: the retention time, easily transformed into the retention volume, if

the flow-rate and the pressure conditions of the column are known. Retention times may be recorded nowadays to 0.01 s, a considerable improvement over the early measurements of the 1950s, when retention times were frequently determined from distances in the recorder chart. Soon after the presentation of GC by James and Martin in 1952 [1] it became evident that reproducibility of retention times was poor, because they depend too much on the particular experimental conditions and thus, have little value for interlaboratory comparisons. Relative retention soon became more promising, as any

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alteration of the chromatographic variables would affect both the analyte and the reference standard in the same way.

An early observation by James and Martin [1] was the fact that the plot of the logarithms of the corrected retention volumes of the members of a homologous series versus their carbon number was a straight line when homologs of at least five or six carbon atoms were considered. The straight line may obviously be observed if the logarithms of the adjusted retention times are plotted versus carbon number (hereafter, the “semilog plot”). Referred to the *n*-alkanes, this fact may be represented by Eq. (1) below:

$$\ln(t_{RZ} - t_M) = B + Cz \quad (1)$$

where *z* represents the number of carbon atoms of the *n*-alkane.

Based on this idea, Kováts proposed to increase the number of reference substances by presenting his retention index system [2]. He defined the retention index of a *n*-alkane as 100 times its carbon number, and that of an analyte eluted isothermally in the same chromatogram as two *n*-alkanes as follows:

$$I_x = 200 \frac{\log V_R^0(x) - \log V_R^0(n - P_z)}{\log V_R^0(n - P_{z+2}) - \log V_R^0(n - P_z)} + 100z \quad (2)$$

where $n - P_z$ and $n - P_{z+2}$ represent the *n*-alkanes of *z* and *z*+2 carbon atoms, *z* being an even number, and V_R^0 represents the corrected retention volume, with compound *x* eluting between the two *n*-alkanes. It was later accepted that any *n*-alkane may be used, and that they may be consecutive (one carbon atom apart). However, presented as the “definition”, this equation rather shows the way by which Kováts proposed that retention indices may be obtained. The real definition of retention index may easily be deduced from the idea that gave rise to the equation: “The retention index of an analyte *x* is equal to 100 times the “apparent” number of carbon atoms of a hypothetical *n*-alkane that would have the same retention time as the analyte *x* under identical chromatographic conditions”. The value of I_x , similar to what happens with the specific retention volume, does not depend on the particular gas chromatograph, the column type or length, the carrier

gas used or its flow-rate. It does depend on the temperature of the column and on the stationary phase. Retention indices have been used for inter-laboratory comparisons, identification of substances and characterisation of stationary phases, among other things [3–9]. They are related to structural and physicochemical properties of both the analyte and the stationary phase. As mentioned above, the normal practice in the calculation of retention indices is to use adjusted retention times, deduced from the experimental retention time (t_R) and the hold-up time (t_M) or retention of an ideal non-retained substance. Decimal or natural logarithms may be used to find I_x . It has also been accepted for a long time that the *n*-alkanes used need not be consecutive.

Retention indices are reported as whole numbers, and according to comments by Blomberg [10] they are normally considered to be obtained with an accuracy no better than one retention index unit (riu) in the case of apolar stationary phases, and lower in the case of polar stationary phases. Blomberg pointed out that with a careful control of the experimental variables these figures could be improved. Reproducibility is related to precision, and precision is related to the chromatographic parameters that control the process: temperature and flow-rate stability and temperature reproducibility. For interlaboratory comparisons, reproducibility of stationary phase characteristics is also important. Present day gas chromatographs offer a high degree of precision of the working variables that control the process (temperature of the oven and flow-rate stability) and a reasonable homogeneity of the oven temperature.

Retention indices were primarily introduced with identification purposes, but the inaccuracy associated with the values obtained by different laboratories, as well as the dramatic decrease in the price of mass spectrometers has brought about a decreasing interest in retention indices. However, quite often mass spectra are inconclusive and the value of the retention index of the compound may help to distinguish between compounds that produce similar mass spectra. In addition, not all laboratories can afford the purchase of a mass spectrometer even with the present day low prices.

For identification purposes and for physicochemical measures affecting either the analyte or the

stationary phase, the accuracy of the retention index is the important magnitude to consider. The retention index of any analyte on a particular stationary phase at a given temperature is one single value, and many scientists think that it is therefore insufficient to unequivocally identify the analyte. However, the exact mass of an ion obtained by high resolution mass spectrometry is another single value, and yet it allows to find the exact atomic composition of the entity better than any other method. The situation in gas chromatography is far from this, but an improvement of the accuracy in the determination of retention indices might bring the technique much closer to an ideal situation. Accepting that precision has reached a reasonable level nowadays, we would have to pay attention to the suitability of the method used to determine retention indices, as the accuracy of the value will depend on the use of the right method under the most appropriate conditions. A higher accuracy in the retention index would certainly represent an important step forward in the use of GC for identification purposes or physicochemical studies, the latter becoming increasingly important in the characterisation of polymers and their blends by inverse gas chromatography (IGC).

According to the definition of retention index given above, the value of I_x must be deduced from the equation that describes the chromatographic behaviour of the n -alkanes, either under isothermal or under programmed temperature conditions. This is equally true for any other retention index system that may be thought of, not necessarily based on the n -alkanes. In that case, the retention index must be deduced from the line or equation that truly describes the chromatographic behaviour of the new reference standards.

Kováts' equation is based on the accepted linear representation of the left-hand side of Eq. (1) versus carbon number of the n -alkanes. It has been claimed that the best way to find retention indices of substances eluted in the same chromatogram as a number of n -alkanes is the use of an iterative method such as that of Guardino et al. [11] to find the equation of the straight line defined by Eq. (1) and then deduce the retention indices of the other analytes from that equation. In this way, experimental retention time variations of the individual n -alkanes would be compensated for. A least mean squares

adjustment of Eq. (1) produces correlation coefficients of the order of 0.99999 or higher, even if n -alkanes of three, four and five carbon atoms are included in the plot [12,13]. However, even if this has been taken as an indication of the linearity of the "semilog plot", the lack of linearity has been mentioned from time to time [14–16], and has been demonstrated with sound proofs [17].

It has been shown [17–19] that the chromatographic behaviour of the n -alkanes under isothermal conditions is better represented by Eq. (3):

$$t_{RZ} = A + \exp(B + Cz^D) \quad (3)$$

This expression is similar to the classical one represented by Eq. (1) with the exception of the superscript D . Parameters A , B and C of Eq. (3) have been shown [20] to have the same relation to chromatographic variables as the corresponding parameters t_M , B and C of Eq. (1). Parameter D , which explains the lack of linearity of the "semilog plot" of the n -alkanes, is independent from any chromatographic variable, being a characteristic of the methylene group. Lebrón-Aguilar et al. [21], comparing results of two iterative methods [11,22] with those based on Eq. (3), have shown that in the case of n -alkanes (the only substances whose retention indices are known beyond doubt), errors of the iterative methods may be as large as 1 riu, depending on the relative position of the analyte inside the range of retention times defined by the n -alkanes of the chromatogram. They have also shown that Eq. (3) of this paper produces retention indices of the n -alkanes much closer to the theoretical value.

It may be claimed that deducing retention indices of n -alkanes from the equation of the line defined by the retention times of the same n -alkanes is not fair. Therefore, we present here results corresponding to other substances eluted in the same chromatogram as a few n -alkanes. Results will be compared with those obtained by an iterative method which relies on Eq. (1). In this paper, only isothermal Kováts' retention indices will be taken into account, but some of the conclusions may be exported to other systems and conditions. In what follows, it will be shown that isothermal retention indices may be obtained with an accuracy of 0.1 riu. This represents an important improvement over present estimations of accuracy.

2. Experimental

Experiments reported in this paper were carried out with a HP-5890A gas chromatograph, fitted with a back pressure regulator (no electronic flow control), split injector and a FID. Column head pressures were monitored with a Wika Tronic 891.13.500 (Alexander Wiegand, Klingenberg, Germany) pressure transducer and a Felix Mateo PM-2900 (Barcelona, Spain) digital indicator with a precision of ± 67 Pa (1 Torr = 133.32 Pa). Nitrogen from a Domnik Hunter gas generator (>99.999% pure) was used as carrier gas.

2.1. Chromatograms

Isothermal chromatograms were obtained on a Zebron ZB-5 capillary column, a poly(95% methyl, 5% phenyl siloxane). Column dimensions were $60 \text{ m} \times 0.25 \text{ mm}$ I.D., with a film thickness of $0.25 \mu\text{m}$. Once the working conditions were achieved, a minimum of 12 h (overnight) was allowed for stability. The injected sample contained the 17 substances shown later.

2.2. Retention indices of analytes

Eqs. (1) and (3) have been used to deduce the values of the parameters t_M , B and C (Eq. (1)), and A , B , C and D (Eq. (3)). In the first case, the iterative method used has been written in FORTRAN in our laboratories [22]. It produces similar results to that of Guardino et al. [11,17]. In the case of Eq. (3), a non-linear regression program written in FORTRAN was used [18,19]. It may be mentioned that the same values of the parameters for Eq. (3) are obtained by using the commercial mathematical packages that present non-linear regressions.

Retention indices of the n -alkanes and other analytes eluted from each chromatogram, deduced from Eq. (1) were calculated as:

$$I_x = 100 \frac{\ln(t_{\text{RX}} - t_M) - B}{C} \quad (4)$$

Retention indices from Eq. (3) were deduced according to the expression:

$$I_x = 100 \sqrt[p]{\frac{\{\ln(t_{\text{RX}} - A)\} - B}{C}} \quad (5)$$

3. Results and discussion

The facts and results presented in this paper have been observed in many chromatograms run after a careful control of all chromatographic variables such as temperature, flow-rate, stability periods, etc. In order to illustrate the recommended method, we present results obtained on one capillary column at one temperature. Results and conclusions are, however, general for capillary columns.

Five chromatograms, each containing nine n -alkanes and eight other substances of different chemical nature, were used to calculate the retention indices of all substances eluted by two different methods. Retention times and the parameters corresponding to Eqs. (1) and (3) are shown in Table 1. It may be worth mentioning the high values of the correlation coefficients of the adjustments of the retention times of the n -alkanes to either equation, although it may be noted that in the case of Eq. (3) they are higher. Retention indices have been calculated by the two methods mentioned so far: Eq. (4) (“linearity method”), and Eq. (5) (“LQG method”). Results of the mean values and the corresponding standard deviations are shown in Table 2. A few conclusions may be derived from the values shown in the table.

Comparing results of the n -alkanes with their theoretical values, it may be seen that retention indices calculated with Eq. (4), based on the linearity of the “semilog plot”, deviate from the expected values by differences ranging from -1.05 to $+0.76$ riu, the error depending on the relative position of the n -alkane within the range of retention times of the chromatogram. The first eluted alkane has no error, because the method based on Eq. (1) has been designed in such a way that the first n -alkane is used as the reference, and its retention index is adjusted to its theoretical value. Values deduced by Eq. (5) are really closer to the expected values, without a clear dependence on the retention time. The standard deviations obtained by the two methods are similar for the compounds eluting in the early part of the chromatogram, but in the “LQG method”, standard

Table 1
Retention times (s) and parameters of Eqs. (1) and (3) for Column ZB-5, 120°C

Substance	Z5120A01	Z5120A02	Z5120A03	Z5120A04	Z5120A05
<i>n</i> -Hexane	161.7	162.1	161.7	161.5	162.3
<i>n</i> -Heptane	170.4	170.9	170.4	170.2	170.9
<i>n</i> -Octane	186.0	186.5	186.0	185.9	186.6
<i>n</i> -Nonane	214.0	214.3	213.9	213.7	214.5
<i>n</i> -Decane	263.5	263.9	263.5	263.3	264.1
<i>n</i> -Undecane	351.6	352.0	351.6	351.4	352.2
<i>n</i> -Dodecane	507.5	507.9	507.4	507.4	508.2
<i>n</i> -Tridecane	782.7	783.1	782.7	782.7	783.6
<i>n</i> -Tetradecane	1267.5	1268.4	1267.5	1267.9	1268.4
1-Chlorobutane	166.3	166.8	166.3	166.1	166.8
<i>n</i> -Pentanenitrile	181.9	182.3	181.8	181.7	182.4
Ethylbenzene	207.0	207.4	207.0	206.8	207.5
2-Octyne	258.5	259.0	258.5	258.3	259.1
2-Nonanone	343.8	344.2	343.7	343.6	344.4
2,6-Dimethylphenol	370.7	371.1	370.6	370.5	371.3
1-Decanol	686.9	687.4	686.7	686.9	687.9
Methyl decanoate	874.6	874.8	874.3	874.6	875.8
Parameters of Eq. (1)					
t_M	150.3942	150.7290	150.4037	150.1843	151.0607
B	-1.0242320	-1.0131394	-1.0256942	-1.0227615	-1.0358133
C	0.5749243	0.5740346	0.5750288	0.5748258	0.5758714
Correlation coefficient	0.9999972	0.9999988	0.9999973	0.9999978	0.9999952
Parameters of Eq. (3)					
A	150.9529	151.3552	150.9763	150.7852	151.6215
B	-1.426825	-1.399914	-1.437038	-1.431239	-1.476918
C	0.702697	0.693687	0.705551	0.703520	0.718106
D	0.942148	0.945848	0.941070	0.941925	0.936179
Correlation coefficient	0.99999995	0.99999994	0.99999998	0.99999996	0.99999995

deviations tend to decrease as the retention times of the analytes increase, while in the case of the “linear method” this is not the case. The reason for this is simple: Eq. (3) defines a curve with a slope that increases with the value of z (carbon number of the n -alkane), therefore a small error in the retention time represents a large change in the estimated retention index in the initial part of the curve (low slope) and a smaller one in the steep part of the curve, at the other end. In the case of a linear adjustment, the effect of an error in the retention time has a similar effect on the retention index, independent of the zone of the plot.

Judging by the values of I_x of the n -alkanes, it might be concluded that the retention indices of the other analytes of the chromatograms, when calculated by Eq. (5), lie within 0.1 riu of their correct

value for the column and conditions of the experiments. Compared with the values deduced by the “linear method”, it may be deduced that the latter is not an appropriate method to find retention indices, even if the values presented in the table fall within 1 riu of the values deduced by the “LQG method”. This is the currently accepted error for retention index accuracy. Results in this paper seem to indicate that accuracy may be considerably improved with present day gas chromatography technology.

3.1. The correctness of the retention indices deduced by the “LQG method”

Parameters A , B , C , and D of Eq. (3) have been deduced from the retention times of the n -alkanes eluted in each chromatogram, and then used to find

Table 2
Retention indices from five chromatograms with Column ZB-5 at 120°C

Substance	From Eq. (4)	From Eq. (5)
<i>n</i> -Hexane	600.00±0.00 ^a	599.96±0.32
<i>n</i> -Heptane	699.26±0.42	699.89±0.38
<i>n</i> -Octane	799.73±0.19	800.08±0.19
<i>n</i> -Nonane	900.23±0.28	900.05±0.13
<i>n</i> -Decane	1000.57±0.20	999.96±0.03
<i>n</i> -Undecane	1100.76±0.12	1100.00±0.02
<i>n</i> -Dodecane	1200.55±0.03	1200.01±0.02
<i>n</i> -Tridecane	1299.94±0.06	1300.00±0.01
<i>n</i> -Tetradecane	1398.95±0.21	1400.00±0.00 ^b
1-Chlorobutane	659.37±0.63	659.90±0.51
<i>n</i> -Pentanenitrile	778.04±0.20	778.48±0.19
Ethylbenzene	880.11±0.18	880.04±0.08
2-Octyne	992.74±0.15	992.15±0.06
2-Nonanone	1093.86±0.12	1093.10±0.03
2,6-Dimethylphenol	1116.51±0.10	1115.76±0.03
1-Decanol	1271.37±0.05	1271.21±0.04
Methyl decanoate	1323.53±0.06	1323.79±0.06

Values are mean value±SD.

^a This *n*-alkane is used as reference, so it has no error.

^b Value lower than 0.005.

the retention indices of all substances in the chromatogram. The good agreement between experimental and theoretical retention indices of the *n*-alkanes is an indication of the accuracy achieved by the method used to find them. However, considering that the previous conclusion has been derived from results of the very substances that were used as reference, its validity might be questioned. In addition, other analytes of the chromatogram present values of the retention index which cannot be confirmed directly.

In order to check the accuracy of the values found for those substances by Eq. (5), five new chromatograms were run, including the same substances of Tables 1 and 2. But in this case, the non-paraffinic substances were used as standards in the determination of the parameters of Eqs. (1) and (3). For this purpose, the retention indices listed in Table 2 deduced by Eq. (5), were divided by 100 and the resulting value used as the “apparent number of carbon atoms” of hypothetical *n*-alkanes. With the values of the parameters thus found, retention indices of all eluted substances were recalculated. Table 3 shows the individual retention times of the five new

chromatograms, and the parameters obtained for Eqs. (1) and (3). Table 4 summarises the retention indices found after the new regression, and the corresponding standard deviations.

Results confirm the conclusions presented previously. Values of the retention indices obtained by the LQG method, of Tables 2 and 4 for the non-paraffinic substances of Table 3 are very close. Retention indices of *n*-alkanes, from Table 4, deduced from the LQG equation derived from the other substances of the chromatogram fall within 0.1 riu of the theoretical value in all except the first two cases. The value of *n*-hexane has been obtained by extrapolation, and this does not seem acceptable towards lower retention times. Extrapolation to higher retention indices is not recommended either, but less than about 100 riu seems a safe distance (see *n*-tetradecane). The case of *n*-heptane will be discussed later. Results confirm the validity of the LQG method for the accurate estimation of retention indices, and the accuracy of the retention indices of the non-paraffinic substances.

3.2. The chromatographic conditions necessary to achieve 0.1 riu accuracy

A check on the chromatographic conditions and number of runs necessary to achieve the 0.1 riu accuracy was carried out with the 10 experiments of Tables 1 and 3. For that purpose, the *n*-alkanes were taken as reference substances to find the parameters of Eq. (3) and the retention indices of all eluted substances recalculated. Mean values of the retention indices deduced from Eq. (5) by successively increasing the number of chromatograms are gathered in Table 5. The table also shows the number of retention index units that correspond to one additional second in the retention time of the corresponding substance (i.e. for *n*-hexane, a retention time of 161.7 s, produces a I_x value of 599.8; with an additional second (162.7 s), the value would be 615; number of riu/s, about 15). Only substances with a retention index below 1200 are shown, because it was observed that above that figure, both accuracy and precision were as good as those shown in Tables 2 and 4.

Two aspects of the table deserve comment. For one part, with the exception of *n*-hexane, 1-chloro-

Table 3
Second set of chromatograms. Retention times (s) and parameters of Eqs. (1) and (3) with Column ZB-5, 120°C

Substance	Apparent number of carbon atoms	Z5120X01	Z5120X02	Z5120X03	Z5120X04	Z5120X05
1-Chlorobutane	6.5990	166.6	166.8	166.8	166.3	166.3
<i>n</i> -Pentanenitrile	7.7848	182.1	182.4	182.5	181.9	181.9
Ethylbenzene	8.8004	207.1	207.6	207.6	207.0	207.0
2-Octyne	9.9215	258.6	259.2	259.3	258.6	258.5
2-Nonanone	10.9310	343.9	344.5	344.8	343.9	343.8
2,6-Dimethylphenol	11.1576	370.8	371.5	371.8	370.8	370.7
1-Decanol	12.7121	686.9	688.2	688.5	687.1	686.9
Methyl decanoate	13.2379	874.8	876.3	876.7	875.1	874.6
<i>n</i> -Hexane	6	162.0	162.3	162.3	161.8	161.7
<i>n</i> -Heptane	7	170.7	171.0	171.0	170.5	170.4
<i>n</i> -Octane	8	186.3	186.6	186.6	186.1	186.0
<i>n</i> -Nonane	9	214.1	214.6	214.6	214.0	214.0
<i>n</i> -Decane	10	263.7	264.3	264.4	263.7	263.5
<i>n</i> -Undecane	11	351.7	352.3	352.6	351.7	351.6
<i>n</i> -Dodecane	12	507.6	508.5	508.8	507.7	507.6
<i>n</i> -Tridecane	13	783.1	784.1	784.8	783.1	782.9
<i>n</i> -Tetradecane	14	1267.9	1269.9	1271.3	1268.4	1268.4
Parameters of Eq. (1)						
t_M		150.6869	150.7358	150.6663	150.2557	150.2567
B		-1.0310298	-1.0134311	-1.0051168	-1.0149141	-1.0143756
C		0.5755678	0.5743332	0.5737273	0.5743704	0.5742792
Correlation coefficient		0.9999982	0.9999990	0.9999991	0.9999989	0.9999990
Parameters of Eq. (3)						
A		151.2450	151.2686	151.2139	150.7060	150.9298
B		-1.420984	-1.342296	-1.334504	-1.317435	-1.408431
C		0.699151	0.676703	0.675703	0.669062	0.696774
D		0.943820	0.952732	0.952953	0.955868	0.944541
Correlation coefficient		0.99999996	1.00000000	0.99999997	0.99999999	1.00000000

butane and perhaps *n*-heptane, there is not a clear indication that an increase in the number of experiments will improve accuracy. In fact, from retention times equal to, or larger than that of *n*-octane, two chromatograms seem sufficient to produce a value which falls within 0.1 riu of the average value from the 10 chromatograms, accuracy increasing with the retention time of the substance. On the other hand, there is a clear relation between the retention times and the accuracy of the retention indices. In order to maintain the 0.1 riu accuracy, retention times should be such that an increase of 1 s in the retention time will represent an increase in the retention index of less than 5 riu. Or expressed in a different way, there must be more than about 30 s between the two consecutive *n*-alkanes that bracket the substance. In

the present case, *n*-octane or ethylbenzene may be considered as the earliest components of the mixture whose retention index fall within the desired limits of accuracy. This is the reason for the inaccuracy of the value of 700.43 ± 0.39 (Table 4) found for *n*-heptane when the non-paraffinic substances are used as references to find the parameters of Eq. (3). Even if the value is found by interpolation, its retention time falls outside the “less than 5 riu/s” rule. Our experience with many other chromatograms run on different stationary phases indicates that a value lower than 5 riu/s is the safe acceptable value to consider.

It may be deduced from the above paragraph that Eq. (4) requires only one chromatogram to obtain retention indices while Eq. (5) requires two. This is

Table 4
Second set of chromatograms. Retention indices from non-paraffinic substances with Column ZB-5 at 120°C

Substance	From Eq. (4)	From Eq. (5)
1-Chlorobutane	659.00±0.00 ^a	660.10±0.20
<i>n</i> -Pentanenitrile	778.16±0.12	778.55±0.13
Ethylbenzene	879.83±0.11	879.90±0.12
2-Octyne	992.39±0.07	992.10±0.04
2-Nonanone	1093.54±0.08	1093.12±0.03
2,6-Dimethylphenol	1116.21±0.06	1115.80±0.02
1-Decanol	1271.08±0.04	1271.19±0.01
Methyl decanoate	1323.33±0.07	1323.80±0.00 ^b
<i>n</i> -Hexane	601.97±1.05 ^c	601.49±1.14 ^c
<i>n</i> -Heptane	700.04±0.39	700.43±0.39
<i>n</i> -Octane	799.66±0.22	800.01±0.20
<i>n</i> -Nonane	900.08±0.11	900.08±0.11
<i>n</i> -Decane	1000.37±0.12	1000.06±0.08
<i>n</i> -Undecane	1100.41±0.08	1100.00±0.03
<i>n</i> -Dodecane	1200.26±0.04	1200.04±0.02
<i>n</i> -Tridecane	1299.73±0.06	1300.03±0.04
<i>n</i> -Tetradecane	1398.83±0.19 ^c	1399.98±0.10 ^c

Values are mean value±SD.

^a This substance is used as reference, so it has no error.

^b Value lower than 0.005.

^c Values obtained by extrapolation.

not the correct interpretation. In fact, either equation produces retention index values from one chromatogram. However, in order to minimise uncertainties, more values are normally required. Five values is the minimum accepted to have a reasonable standard deviation. What this paper shows is that in the case of Eq. (5) two chromatograms are sufficient to have a value within 0.1 riu of the correct one, as long as the “less than 5 riu/s rule” is observed. However, Eq. (4) will not give an accurate value in any case, deviating from the real value more or less, depending on the relative position of the compound within the range covered by the *n*-alkanes used. Therefore, more injections will minimise uncertainty (precision) but will never approach the average value for the accurate retention index of the compound.

3.3. The effect of the time base on the accuracy and precision of retention indices

In order to achieve the accuracy of 0.1 riu, it is necessary to pay attention to the time base used to measure retention times. Results presented so far

Table 5
Effect of the number of chromatograms on retention index accuracy

Number of runs	<i>n</i> -Hexane	<i>n</i> -Heptane	<i>n</i> -Octane	<i>n</i> -Nonane	<i>n</i> -Decane	<i>n</i> -Undecane
2	599.79±0.40	700.15±0.37	800.00±0.21	900.06±0.24	999.93±0.00	1100.00±0.02
3	599.88±0.32	700.09±0.29	799.98±0.16	900.05±0.17	999.95±0.04	1100.01±0.02
4	599.87±0.27	700.02±0.27	800.08±0.22	900.03±0.16	999.95±0.03	1100.00±0.02
5	599.96±0.32	699.89±0.38	800.08±0.19	900.05±0.14	999.95±0.03	1100.00±0.02
6	599.85±0.39	699.90±0.34	800.10±0.18	900.05±0.12	999.98±0.05	1099.99±0.02
7	599.92±0.39	699.90±0.31	800.05±0.21	900.04±0.12	999.99±0.06	1099.98±0.03
8	600.01±0.45	699.91±0.29	800.00±0.24	900.05±0.11	1000.01±0.07	1099.99±0.03
9	600.03±0.42	699.91±0.27	799.99±0.22	900.04±0.11	1000.01±0.07	1099.98±0.03
10	600.08±0.41	699.91±0.25	799.97±0.22	900.00±0.11	1000.01±0.07	1099.98±0.03
riu/s	15	8.5	4.8	2.7	1.5	0.9
	1-Chlorobutane	<i>n</i> -Pentanenitrile	Ethylbenzene	2-Octyne	2-Nonanone	2,6-Dimethylphenol
2	660.27±0.48	778.57±0.15	880.01±0.06	992.17±0.11	1093.12±0.02	1115.79±0.02
3	660.17±0.38	778.45±0.23	880.05±0.06	992.16±0.08	1093.10±0.04	1115.77±0.04
4	660.08±0.36	778.50±0.22	880.06±0.07	992.15±0.07	1093.10±0.03	1115.77±0.03
5	659.90±0.51	778.48±0.19	880.04±0.08	992.15±0.06	1093.10±0.03	1115.76±0.03
6	659.88±0.46	778.48±0.17	880.00±0.11	992.14±0.07	1093.10±0.02	1115.76±0.02
7	659.74±0.56	778.42±0.23	879.99±0.10	992.13±0.06	1093.09±0.03	1115.76±0.02
8	659.65±0.57	778.43±0.21	879.97±0.12	992.13±0.06	1093.10±0.03	1115.77±0.03
9	659.57±0.58	778.40±0.22	879.94±0.13	992.13±0.06	1093.10±0.03	1115.77±0.03
10	659.61±0.57	778.42±0.21	879.95±0.12	992.12±0.05	1093.10±0.03	1115.77±0.03
riu/s	10.6	5.4	3	1.6	0.9	0.3

Chromatograms from Tables 1 and 3. Eq. (3) adjusted to the *n*-alkanes.

have been obtained measuring retention times to 0.1 s, but sometimes, integrators and computer programs present retention times in minutes. For the purpose of comparing results, retention times as presented in Table 1 were recalculated to minutes, rounding off results to 0.01 and 0.001 min. The new retention times were used again to calculate results equivalent to those of Table 2. Average retention indices and the corresponding standard deviations are shown in Table 6.

Retention indices calculated from retention times recorded to 0.001 min are very close to those calculated when they are measured to 0.1 s. Differences in the *n*-alkanes are of 0.05 riu in two cases, and 0.02 or lower in all others. For other analytes eluted in the same chromatograms, differences are 0.02 riu or lower. It may be concluded that recording retention times with an accuracy of 0.001 min is as safe as doing it to 0.1 s. But the use of 0.01 min will assure the 0.1 riu accuracy only for those retention times for which an additional minute will represent about less than 75 riu, as may be observed for *n*-decane in Table 6. The parameters of the equation for chromatogram Z5120A01 of Table 1, when recorded to 0.01 min are: $A=2.5228$; $B=-$

5.627870 ; $C=0.734429$; $D=0.930165$. A retention time of 4.39 min, produces a retention index of 999.82; with 5.39 min, it becomes 1073.74.

4. Conclusions

Gas chromatographic isothermal Kováts' retention indices of substances eluted from a capillary column may be obtained with an accuracy of 0.1 riu by the use of Eq. (3) of this paper, applying a non-linear regression procedure. This accuracy will only be possible if retention times are measured to 0.1 s or 0.001 min, and besides that, they should be such that an additional second in the retention time would represent an increase of between 2 and 3 riu. Longer retention times are needed if they are measured to 0.01 min with the minimum corresponding to a retention time for which an additional minute would represent less than 75 riu.

The average values from two chromatograms of the same sample should produce results with an accuracy of 0.1 riu with present day gas chromatographs, without electronic flow controller, as long as sufficient time is allowed for stabilisation.

Table 6
Retention indices calculated with Eq. (5) from retention times (min)

Substance	Minutes as X.XX mean value \pm SD	Minutes as X.XXX mean value \pm SD	Approx. riu/min ^a
<i>n</i> -Hexane	600.16 \pm 2.01	599.97 \pm 0.43	319
<i>n</i> -Heptane	699.73 \pm 1.47	699.84 \pm 0.37	241
<i>n</i> -Octane	799.95 \pm 0.61	800.06 \pm 0.20	171
<i>n</i> -Nonane	900.26 \pm 0.69	900.10 \pm 0.12	115
<i>n</i> -Decane	999.87 \pm 0.08	999.97 \pm 0.05	74
<i>n</i> -Undecane	1100.00 \pm 0.03	1100.00 \pm 0.03	45
<i>n</i> -Dodecane	1200.02 \pm 0.05	1200.00 \pm 0.02	27
<i>n</i> -Tridecane	1300.00 \pm 0.03	1300.00 \pm 0.01	16
<i>n</i> -Tetradecane	1400.00 \pm 0.00 ^b	1400.00 \pm 0.00 ^b	9
1-Chlorobutane	658.45 \pm 2.06	659.90 \pm 0.61	270
<i>n</i> -Pentanenitrile	778.19 \pm 0.74	778.47 \pm 0.22	184
Ethylbenzene	880.20 \pm 0.29	880.06 \pm 0.13	125
2-Octyne	992.34 \pm 0.06	992.14 \pm 0.09	76
2-Nonanone	1093.13 \pm 0.03	1093.10 \pm 0.04	47
2,6-Dimethylphenol	1115.86 \pm 0.03	1115.75 \pm 0.03	42
1-Decanol	1271.22 \pm 0.09	1271.21 \pm 0.04	19
Methyl decanoate	1323.78 \pm 0.10	1323.80 \pm 0.06	14

Data from Table 1.

^a Retention index units corresponding to one additional minute.

^b Values lower than 0.005.

Parameters of Eq. (3) may be obtained from the retention times of a minimum of four *n*-alkanes not necessarily consecutive, or four other substances whose retention indices had been previously measured with sufficient accuracy. Any commercial mathematical package that contains non-linear regression may be used.

Extrapolation to find retention indices of substances eluted outside the time range defined by the standards used (*n*-alkanes or other substances) is not allowed towards shorter retention times, and is not recommended at larger retention times beyond, say, 100 ri.

Injection of a hold-up time indicator, or any other way of calculating its value by a mathematical procedure is not necessary if Eq. (3) of this paper is used.

It is once more confirmed that the “semilog plot”, even with correlation coefficients as high as 0.999995 is not a straight line, not representing the chromatographic behaviour of the *n*-alkanes under isothermal conditions. Therefore, the use of the “semilog plot” (the linear approach) does not guarantee the accuracy of 0.1 ri.

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References

- [1] A.T. James, A.J.P. Martin, *Biochem. J.* 50 (1952) 679.
- [2] E.S. Kováts, *Helv. Chim. Acta* 41 (1958) 1915.
- [3] W.O. McReynolds, *J. Chromatogr. Sci.* 8 (1970) 685.
- [4] G. Schomburg, G. Dielmann, *J. Chromatogr. Sci.* 11 (1973) 151.
- [5] L.S. Ettre, *Chromatographia* 6 (1973) 489.
- [6] 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.
- [7] 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.
- [8] V. Pacáková, L. Felzl, *Chromatographic Retention Indices. An Aid to Identification of Organic Compounds*, Ellis Horwood, Chichester, 1992.
- [9] C.T. Peng, *J. Chromatogr. A* 678 (1994) 189.
- [10] L.G. Blomberg, *Adv. Chromatogr.* 28 (1987) Chapter 6.
- [11] X. Guardino, J. Albaigés, G. Firpo, R. Rodríguez-Viñals, M. Gassiot, *J. Chromatogr.* 118 (1976) 13.
- [12] L.S. Ettre, *Chromatographia* 13 (1980) 73.
- [13] M.R. Becerra, E. Fernández-Sánchez, J.A. García-Domínguez, J. García-Muñoz, M.J. Molera, *J. Chromatogr. Sci.* 20 (1982) 363.
- [14] L. Rohrschneider, *Chromatographia* 2 (1969) 437.
- [15] R.V. Golovnya, D.N. Grigoryeva, *Chromatographia* 17 (1983) 613.
- [16] A. Touabet, M. Maeck, A.Y. Badjah Hadj Ahmed, B.Y. Meklati, *Chromatographia* 25 (1988) 389.
- [17] J.A. García-Domínguez, J.E. Quintanilla-López, R. Lebrón-Aguilar, *J. Chromatogr. A* 803 (1998) 197.
- [18] R. Lebrón-Aguilar, J.E. Quintanilla-López, J.A. García-Domínguez, *J. Chromatogr. A* 760 (1997) 219.
- [19] J.E. Quintanilla-López, R. Lebrón-Aguilar, J.A. García-Domínguez, *J. Chromatogr. A* 767 (1997) 127.
- [20] J.E. Quintanilla-López, R. Lebrón-Aguilar, J.A. García-Domínguez, *J. Chromatogr. A* 878 (2000) 125.
- [21] R. Lebrón-Aguilar, J.A. García-Domínguez, J.E. Quintanilla-López, *J. Chromatogr. A* 805 (1998) 161.
- [22] J.A. García-Domínguez, J. García-Muñoz, E. Fernández-Sánchez, M.J. Molera, *J. Chromatogr. Sci.* 15 (1977) 520.