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# ACCURACY IN THE DETERMINATION OF THE KOVÁTS RETENTION INDEX

## MATHEMATICAL DEAD TIME

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## SUMMARY

The influence of several parameters on the viability and precision of the determination of the Kováts retention index, under conventional experimental conditions, has been examined. In particular, the activity of the support and the dead-time correction have been considered. A new mathematical method for the calculation of the dead time is given.

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## INTRODUCTION

Retention indices, particularly that defined by Kováts[1], are widely used in gas chromatography (GC), since they permit a good way of comparing the chromatographic retention data, as well as the establishment of a series of correlations between retention data and the physical[2–4] or structural properties[5–8] of the separated compounds. However, the accuracy of the determination of retention indices is subject to certain instrumental limitations or sources of error, as shown by the correlation trials carried out in different laboratories[9,10]. Sources of error that have been found to influence the precision and accuracy are: flow-rate and column temperature[10–12]; purity of the mobile and stationary phases[13–17]; activity of the support[18–21]; column efficiency[12,19]; sample size[12,19]; retention measurements and calculations (dead time[22–24], extrapolation, etc.).

Since the real value of the indices is unknown, the accuracy of the results can only be checked by determining their precision and their reproducibility. Chromatographic apparatus has been designed in order to obtain measurements of high precision[25–27]. Most of the GC analyses are carried out with commercial instruments, and the present paper deals with the evaluation of instrumental parametres that have the main influence on the reliability of the retention indices, in particular the activity of the support and the dead time[18]. The prediction of the scatter of the results, through a study of the errors in measurement and their transmission through the calculation process, is examined. A new method for the calculation of the dead time, through iteration, is also given.

## CALCULATIONS

The retention index may be calculated by numerical interpolation, by means of the general equation proposed by Kováts[1],

$$I = 100\left(n + \frac{\log t'_x - \log t'_n}{\log t'_{n+1} - \log t'_n}\right) \tag{1}$$

(where $t'_x$ is the corrected retention time of the problem, $t'_n$ and $t'_{n+1}$ are the corrected retention times of the *n*-alkanes used ($t'_n < t'_x < t'_{n+1}$), $I$ is the retention index, and $n$ is the carbon number of the first alkane used), or by graphical interpolation within the linear relation of $\log t'_R$ and the number of carbon atoms[23,24], $n_C$, according to

$$\log t'_R = aI + b \tag{2}$$

where $t'_R$ is the corrected retention time, and $a$ and $b$ are constants.

Although the first of these methods seems to be simple, the second method is less subject to error, since least-squares treatment of the straight line represented by eqn. 2 results in retention data with an uncertainty which is determined by the precision of the instruments used. The larger the number of experimental points which lie on the straight line, the smaller is the uncertainty in the least-squares results. It can be seen that, with this method, unknown values may be calculated by extrapolation.

The value of the dead time, $t_m$, affects the calculation of the corrected retention time, and its accuracy therefore affects the least-squares adjustment and hence the accuracy of the resulting retention indices. The accepted method of calculation of $t_m$ is that proposed by Haferkamp[22] and later justified by Groenendijk and Van Kemenade[23], where the approximate retention time is represented by three exponential terms:

$$t_m = \frac{t_{n-i} \cdot t_{n-i} - t_n^2}{t_{n+i} + t_{n-i} - 2t_n};\ i = 1, 2, 3, \text{etc.} \tag{3}$$

However, because of the linear relation between $\log t'_R$ and $n_C$ (refs. 23 and 24) it is also possible to calculate a value of $t_m$, which we term the "mathematical dead time", such that the adjustment of the experimental points to the straight line is optimized. An iterative method of calculation has been developed in which the squares of the deviations of the theoretical indices of *n*-alkanes are minimized with respect to the index calculated from eqn. 2. The corresponding flow diagram is shown in Fig. 1.

The error inherent to the calculation of the retention index may be obtained from

$$I = \frac{\log (t_R - t_m) - b}{a} \tag{4}$$

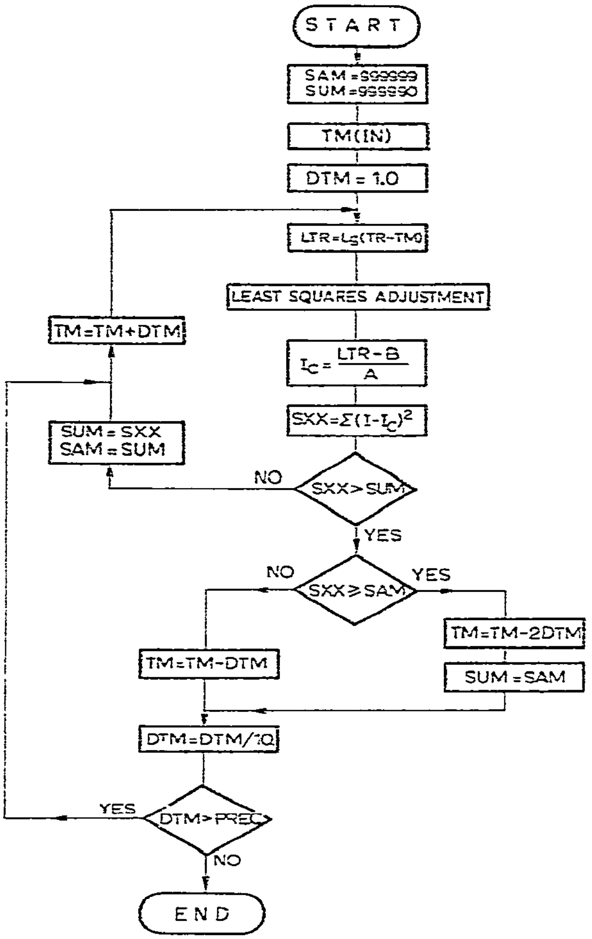

Fig. 1. Routine flow diagram for the calculation of the mathematical dead time. TM (IN) is an initial value of $t_m$, estimated as 90% of Haferkamp's $t_m$ value[22]. A and B are constants, TM is the actual value of the mathematical dead time, DTM is the increased value of TM, TR is the retention time, LTR is the logarithm of the corrected retention time, and PREC is the approximation level of TM-value calculations.

where $a$ and $b$ are the slope and the intercept of the straight line, which is adjusted by least-squares treatment to the retention data of all of the $n$-alkanes used. The absolute errors in $a$ and $b$ are assimilated with the reliability limits obtained from the least-squares expression, for a 95% confidence limit. The statistical error, $\varepsilon_I$, in the calculated retention index may be obtained from

$$\varepsilon_I = \frac{0.43429}{a\,(t_R - t_m)} \cdot (\varepsilon_{t_R} + \varepsilon_{t_m}) + \frac{1}{a} \cdot \varepsilon_b + \frac{\log\,(t_R - t_m)}{a^2}\,\varepsilon_a \qquad (5)$$

where

$$\varepsilon_b = t_{\alpha/2,\ (N-2)} \sqrt{\frac{\Sigma\,(\log t'_{R(i)} - \log t'_{R(i)\ \mathrm{calc}})}{N-2}} \cdot \left(\frac{1}{N} + \frac{\bar{I}^2}{\Sigma\,(I_{(i)} - \bar{I})^2}\right) \qquad (6)$$

$$\varepsilon_a = t_{\alpha/2\ (N-2)} \sqrt{\frac{\Sigma\,(\log t'_{R(i)} - \log t'_{R(i)\ \mathrm{calc}})^2}{(N-2)\,\Sigma\,(I_{(i)} - \bar{I})^2}} \qquad (7)$$

and where $t_{R(i)}$ is the retention time of hydrocarbon $i$; $t_{R(i)\mathrm{calc}}$ is the retention time of hydrocarbon $i$ calculated from eqn. 2; $I_{(i)}$ is the retention index of hydrocarbon $i$; $\bar{I}$ is the mean value of the indices of the hydrocarbons used; and $N$ is the total number of hydrocarbons used. $t_{\alpha/2,\,(N-2)}$ is the value of the Student function at $(1 - \alpha)$ % confidence level, for $N - 2$ degrees of freedom ($N$ is the total number of points). The value of $\varepsilon_I$ obtained from eqn. 5 will normally be higher than the real error in the retention index, which could be calculated only if the value of the index was known; however, the magnitude of $\varepsilon_I$ reflects the reliability that can be placed on such a numerical result.

## RESULTS AND DISCUSSION

### *Correction for the dead time*

The easiest way of evaluating the influence of the method of calculation of the dead time on the accuracy of the retention index is to use different values of the dead time and then compare the values of the retention index which result with the

TABLE I

INFLUENCE OF THE METHOD OF CALCULATION OF THE DEAD TIME ON THE ACCURACY OF THE RETENTION INDEX

| *Theoretical retention time* | *Retention time* | *Calculated retention index* | | |
|---|---|---|---|---|
| | | $I_1$ | $I_2$ | $I_3$ |
| (a) Theoretical retention times ("ideal" straight line) | | | | |
| 2000 | 20.13 | 1999.95 | 1999.99 | 1999.99 |
| 2400 | 48.78 | 2400.04 | 2400.00 | 2400.00 |
| 2800 | 133.14 | 2800.03 | 2799.99 | 2799.99 |
| 3000 | 224.60 | 3000.01 | 2999.99 | 2999.99 |
| 3200 | 381.55 | 3200.00 | 3199.99 | 3199.99 |
| 3600 | 1113.05 | 3599.97 | 3600.00 | 3600.00 |
| $t_m$ | | 5.40 | 5.39 | 5.39 |
| Relative mean deviation | | $0.120 \cdot 10^{-2}$ | $0.140 \cdot 10^{-3}$ | $0.121 \cdot 10^{-3}$ |
| Confidence limit for $a$ | | $0.194 \cdot 10^{-6}$ | $0.364 \cdot 10^{-7}$ | $0.327 \cdot 10^{-7}$ |
| Confidence limit for $b$ | | $0.842 \cdot 10^{-3}$ | $0.104 \cdot 10^{-3}$ | $0.934 \cdot 10^{-4}$ |
| Regression coefficient, square | | 0.9999999 | 1.0000000 | 1.0000000 |
| (b) Practical retention times (one run; stainless-steel capillary column; 30 m × 0.3 mm I.D.; stationary phase, Apiezon L; temperature, 120°) | | | | |
| 700 | 254.0 | 721.37 | 693.18 | 700.67 |
| 800 | 271.0 | 799.93 | 798.49 | 798.45 |
| 900 | 301.0 | 891.02 | 903.15 | 899.82 |
| 1000 | 352.5 | 989.03 | 1005.28 | 1001.17 |
| 1100 | 440.0 | 1090.75 | 1105.19 | 1101.70 |
| 1200 | 580.0 | 1190.00 | 1199.35 | 1197.17 |
| 1300 | 839.0 | 1299.54 | 1301.0 | 1300.83 |
| 1400 | 1267.0 | 1405.95 | 1398.68 | 1400.51 |
| 1500 | 1990.0 | 1512.35 | 1495.54 | 1499.63 |
| $t_m$ | | 218.03 | 232.17 | 229.16 |
| Relative mean deviation | | 0.901 | 0.338 | 0.104 |
| Confidence limit for $a$ | | $0.182 \cdot 10^{-3}$ | $0.738 \cdot 10^{-4}$ | $0.247 \cdot 10^{-4}$ |
| Confidence limit for $b$ | | 0.206 | 0.0834 | 0.0279 |
| Regression coefficient, square | | 0.998284 | 0.999772 | 0.999973 |

theoretical values. A set of theoretical data for *n*-alkanes was chosen so as to overcome the uncertainty due to experimental error (Table Ia). The retention index was determined using (i) a value of the dead time calculated from eqn. 3 using the last three hydrocarbons ($I_1$), (ii) a value of the dead time calculated from eqn. 3 taking the largest possible even spacing (C$_{20}$, C$_{28}$, C$_{36}$) ($I_2$) (iii) the mathematical dead time using all the hydrocarbons ($I_3$). It can be seen from Table Ia that the three methods are nearly equivalent, the optimum result being obtained by methods (ii) and (iii). In Table Ib the same methods were used for some experimental data. In this case the best calculation of the retention index was achieved by use of a more exact calculation of the dead time, as in method (iii). When eqn. 3 was used for the calculation of the dead time, the results were always more precise if the three hydrocarbons which were selected had the largest possible even spacing, starting from the last hydrocarbon in the series (Table II).

TABLE II

INFLUENCE OF EVEN SPACING ON THE CALCULATION OF $t_m$ ACCORDING TO HAFERKAMP (EQN. 3)

Alkanes from *n*-heptane to *n*-pentadecane were used. Mathematical dead time, 403.7.

| *n* | *i = 1* | *i = 2* | *i = 3* | *i = 4* |
|---|---|---|---|---|
| 8 | 407.0 | | | |
| 9 | 400.1 | 403.3 | | |
| 10 | 402.5 | 401.8 | 403.2 | |
| 11 | 403.8 | 403.1 | 402.2 | 403.1 |
| 12 | 402.7 | 402.8 | 402.6 | |
| 13 | 400.9 | 401.2 | | |
| 14 | 399.0 | | | |
| Mean | 402.3 | 402.4 | 402.7 | 403.1 |

The influence of the dead time on the measurement and calculation of the retention index and the interdependence between the precision of the measurement and the capacity factor ($k'$) of the solute studied are related. This correlation is presented in Fig. 2, where the absolute statistical error for a set of theoretical retention-index data is shown as a function of the dead time and of the capacity factor. So, in order to obtain reliable measurements of retention time, the chromatographic conditions should be chosen so as to provide a high capacity factor and also a high value of the dead time (capillary columns).

### *Extrapolation*

The linearization of the relation between log $t'_R$ and $n_C$ permits retention indices to be obtained by extrapolation over a range of alkanes. The reliability of this process may be studied on the basis of two variable factors: the scatter of the points about the adjusted straight line and the distance of extrapolation, that is the difference between the last standard alkane and the alkane whose retention index is required. As far as the first factor is concerned, it can be seen from Fig. 3 that when the mean relative error of the points in the straight line increases, the absolute error of the retention index, $\varepsilon_e = I_{ext} - I_{int}$, increases linearly. With respect to the second factor, $\varepsilon_e$ also increases with increasing extrapolation distance, depending on the capacity factor of the last standard alkane used. In cases of well adjusted straight lines,

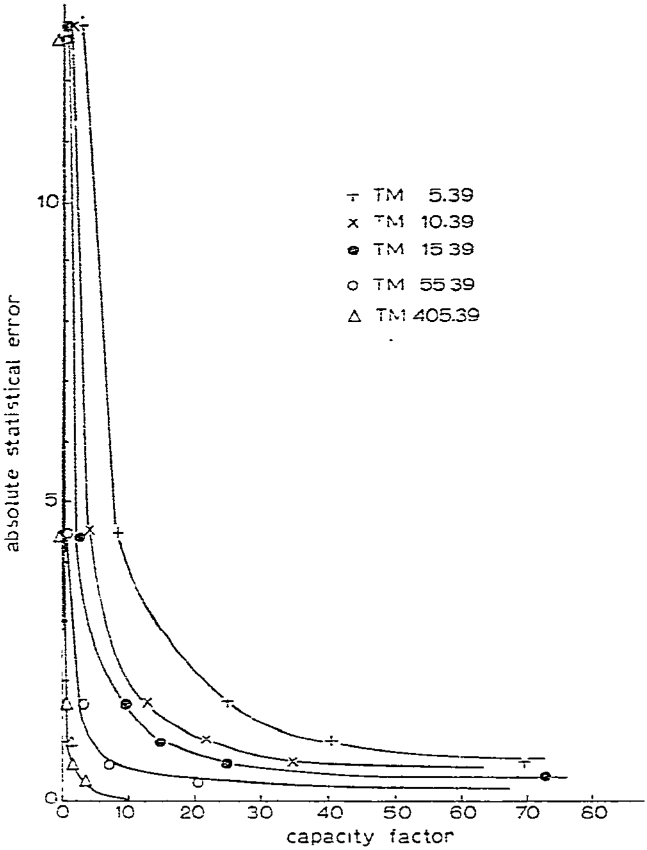

Fig. 2. Variation of absolute statistical error in the retention index as a function of the dead time and the capacity factor.

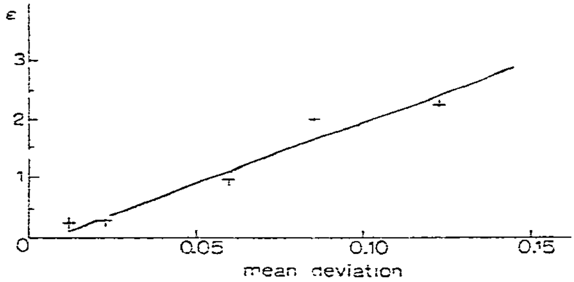

Fig. 3. Absolute error of the retention index as a function of the relative mean deviation of points from the straight line.

the largest extrapolation distance that can be used may be about three times the capacity factor of the last standard alkane (Table III).

*Influence of the activity of the support and the sample size*

The activity of the support is usually responsible for the variation in the reten-

TABLE III

EXTRAPOLATION ERROR AS A FUNCTION OF THE CAPACITY FACTOR OF THE UNKNOWN ALKANE ($k'$) AND OF THE LAST STANDARD ALKANE ($k'_n$), FOR DIFFERENT RELATIVE MEAN DEVIATIONS FROM THE STRAIGHT LINE

| $k'_n$ | $k'$ | | | | | |
|---|---|---|---|---|---|---|
| | *2* | *4* | *6* | *8* | *10* | *15* |
| Mean deviation, 0.12% | | | | | | |
| 0.9 | 2.5 | 4.7 | 6.5 | 8.0 | 8.8 | 10.5 |
| 1.6 | 0.9 | 2.3 | 3.8 | 4.9 | 5.5 | 6.8 |
| 2.7 | | 0.9 | 2.0 | 3.0 | 3.4 | 4.4 |
| 4.5 | | | 1.0 | 1.8 | 2.0 | 2.9 |
| 7.5 | | | | 0.8 | 1.0 | 1.6 |
| 13.0 | | | | | | 0.6 |
| Mean deviation, 0.02% | | | | | | |
| 1.1 | 0.3 | 0.5 | 0.5 | 0.7 | 1.0 | 1.8 |
| 1.7 | 0.1 | 0.2 | 0.2 | 0.3 | 0.7 | 1.0 |
| 2.8 | | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 |
| 4.5 | | | 0.1 | 0.1 | 0.1 | 0.1 |
| 7.2 | | | | 0.0 | 0.1 | 0.0 |
| 11.6 | | | | | | 0.0 |

tion time with the sample size. By using open capillary columns, the effect of the support may be reduced to that of the column wall. This effect may explain the different behaviour of benzene and *n*-heptane when decreasing amounts of sample are injected into stainless-steel and glass-capillary columns (Fig. 4). Schomburg[19] had shown previously the influence of the different polarity of glass and steel on the retention indices

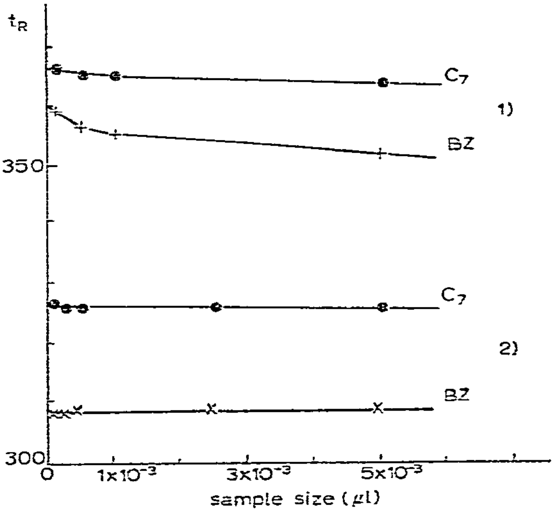

Fig. 4. Variation of retention time with the amount of sample. 1, Stainless-steel capillary column (30 m × 0.3 mm I.D.). Stationary phase, Apiezon L. Temperature, 80°. 2, Capillary column of borosilicate glass. Conditions as in 1.

of alkanes. This phenomenon is intensified with the progressive exhaustion of the column, producing an increase in the retention index which is proportional to the polarity of the chromatographed compound, for the same amount of injected sample (Table IV).

TABLE IV

VARIATION OF THE RETENTION INDEX WITH PERIOD OF USE OF THE COLUMN
Stainless-steel column (30 m × 0.3 mm I.D.); Apiezon L; temperature, 120°.

| *Compound* | *Period of column use (h)* | | |
|---|---|---|---|
| | *0* | *400* | *1200* |
| Benzene | 693.1 | 693.9 | 707.6 |
| Toluene | 800.5 | 801.1 | 809.9 |
| *p*-Xylene | 904.3 | 904.6 | 908.0 |
| Cumene | 943.2 | 943.1 | 945.2 |
| Cyclohexane | 693.3 | 693.6 | 696.3 |
| Methylcyclohexane | 756.2 | 756.4 | 758.0 |

Use of an excess of the injected sample causes asymmetry in the resulting peaks and displacement of their maxima, falsifying the measurement of the retention time. Furthermore, the amount of sample influences the efficiency of the chromatographic system[11,28] as shown in Fig. 5, the optimum sample size being obtained in the absence of solute–support interactions[29].

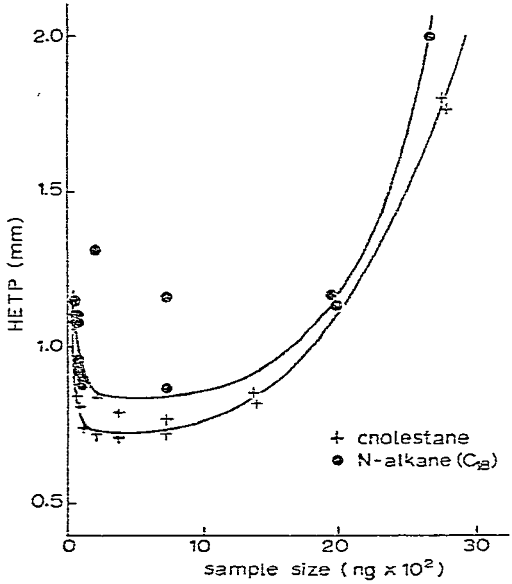

Fig. 5. Dependence of the efficiency on the sample size. All-glass system; column, 27 m × 0.3 mm I.D. Stationary phase, SE-30. Temperature, 220°.

## CONCLUSIONS

The most precise method of calculation of the retention index is through eqn. 2 whose parameters *a* and *b* are adjusted by the method of least squares to the whole of the experimental retention data for a series of *n*-alkanes. Moreover, the larger the number of *n*-alkanes used (usually from six to ten) and the greater the number of times the analysis is repeated, the greater will be the reliability of the indices. However, use of the "mathematical dead time" results in more accurate results and enables the number of runs to be reduced. The linearity of the logarithmic plot, eqn. 2, has been demonstrated to a confidence limit of better than 99.99% in the $C_7$–$C_{36}$ range. With lower data dispersions, the mathematical and conventional methods (eqn. 3) yield equivalent results.

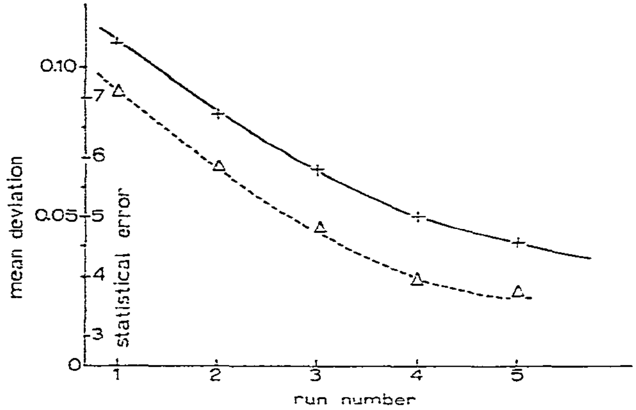

Fig. 6. Dependence of error on the number of runs. — — —, Relative mean deviation from the straight line; ———, absolute statistical error.

The effect of the number of analyses on the reliability of the calculated retention index is shown in Fig. 6. Both graphs show that when the number of assays is larger than four, reliable results are obtained. Once a good constancy of instrumental factors has been obtained (0.1°, 0.03 atm), there is very little difference between the retention indices calculated from data obtained from joint or separate elution of the standard and unknown alkanes (Table V). Although in the case of joint elution a slight improvement in the precision is obtained, some difficulties may arise due to overlapping of peaks.

TABLE V

VARIATION OF THE RETENTION INDEX AND ITS DISPERSION ERROR FOR JOINT ($I_j$) AND SEPARATED ELUTION ($I_s$) OF STANDARD AND UNKNOWN ALKANES

Stainless-steel capillary column (30 m × 0.3 mm I.D.); stationary phase, Apiezon L.

| *Compound* | $I_s$ | $\varepsilon_s$ | $I_j$ | $\varepsilon_j$ |
|---|---|---|---|---|
| Benzene (70°) | 684.5 | 0.82 | 684.4 | 0.77 |
| Toluene (80°) | 794.6 | 0.60 | 794.1 | 0.50 |
| *p*-Xylene (100°) | 904.1 | 0.66 | 904.4 | 0.34 |

The control of the parameters detailed in the present work has permitted the determination of retention indices of saturated, olefinic and aromatic hydrocarbons on a conventional gas chromatograph, with relative errors of between 0.01 and 0.05%.

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