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PRECISION OF GAS CHROMATOGRAPHIC RETENTION DATA IN THE MANUAL PROCESSING OF THE CHROMATOGRAM

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SUMMARY

Absolute and relative retention data and retention indices used for the quali‾ tative characterization of substances analyzed in gas chromatography are not con‾ stant quantities, but quantities characterized by certain variances. A linear rela‾ tionship has been found between the standard deviation of corrected absolute reten- tion data and their magnitudes, the numerical expression of which can be looked upon as characteristic for the given type of substance analyzed, the stationary phase used and for the experimental conditions employed. Some questions concerning the calculation of the relative retention data and retention indices from absolute retention data are discussed on the basis of the above-mentioned empirical relation. It is pos- sible to estimate, from the variance of absolute retention data obtained experimen- tally, the standard deviation of relative retention data and retention indices of the substances analyzed, the variances calculated being in a good agreement with those obtained experimentally. The standard deviation then characterizes, in a useful manner, the precision of the retention data of the substance analyzed, obtained under given conditions.

INTRODUCTION

The increasing number of published gas chromatographic data for substances analyzed on various stationary phases has brought forth a number of proposals for unifying the type of gas chromatographic retention data published. It has been sug- gested by the authors of "Recommendations for the Publication of Retention Data"[1] that the retention data should be published, for common analytical purposes, in the form of Kováts indices I (ref. 2) whenever it is possible, or in the form of relative retention data R (ref. 3) related to n-nonane[4], either directly or by means of a sec- ondary standard. The unification of the type of retention data published, the definition of the way they were obtained from the basic data measured, and a statement con- cerning the experimental conditions (the data are only given their full value on quota- tion of the latter)[1,5] were the first steps towards the utilization of the published retention data obtained from qualitative gas chromatography as a means of identifying the substances analyzed.

With the same purpose in view, some authors[1,6-8] evaluated the reproducibility of retention data measured for substances of different chemical character on various stationary phases, various instruments, and in different laboratories, and the results of these measurements were processed, to different extents, statistically.

ADLARD et al.[1] have recommended that the retention index I is used as a qualitative characteristic, as the percentage standard deviation displayed by retention indices of substances analyzed on squalane as a stationary phase were lower than those of relative retention data of the type R_{xN}, R_{x9}, and R_{xS} (related to a hydrocarbon with N and nine carbon atoms, and to a standard substance S, respectively).

The members of the Data Subcommittee of the GC Discussion Group[6] have measured the retention indices of an identical group of substances at different laboratories, and found a mean deviation of ± 1 unit on squalane and dinonylphthalate as stationary phases, and a mean deviation of ± 3 units on PEG 400. When PEG 400 coming from different batches is used, the mean deviation of the retention indices rose to ± 5 units. It was also found, from the same series of measurements, that the mean deviation of retention indices measured on polar stationary phases depends on the amount of sample injected. On the basis of reviewing retention indices measured on PEGA in "The Fatty Acids Ester — Hydrocarbon Correlation Trial", SWOBODA[7] recommended the use of secondary standards similar in chemical nature to the substances analyzed as a basis for a logarithmic scale to increase the precision of measuring the retention indices of polar substances.

A very detailed statistical treatment of the repeatability and reproducibility of retention indices as well as the respective results have been described in a paper by LOEWENGUTH[8]. He concludes by recommending that when publishing or using retention indices in qualitative analysis, the basic factors contributing to the variance of the retention indices should be taken into consideration, namely, the measurement of the retention time and column temperature control (the second factor plays a significant role particularly with substances showing higher $\Delta I / 10$ values).

The list of papers giving more or less consistent data on the reproducibility of measuring retention data under conventional analytical conditions can be supplemented by papers devoted to the precision of measuring retention data at programmed temperatures[9,10]. In addition, data have been published on the precision of measuring retention indices under experimental conditions which have been arranged to give very precise measurements[11-14].

The aim of the present paper is to complete the existing information on the reproducibility of retention data measured under current analytical conditions by an analysis of the repeatibility of basic types of retention data measured under simplified conditions which eliminate such effects as the differences in the nature of solute and sorbents, chemical nonuniformity of the stationary phase, sample size, incomplete separation of the substances, and sorption activity of the support. The conclusions derived may assist in the correct handling of retention data in qualitative gas chromatography.

EXPERIMENTAL

The absolute retention data of basic types of hydrocarbons were measured on a Shimadzu GC-4A PTF apparatus, employing the variant with thermal conductivity

detection. 1.0 µl of a hydrocarbon mixture (n-alkanes: n-pentane, n-hexane, n-heptane, n-octane; alicyclic hydrocarbons: cyclopentane, cyclopentene, cyclohexane, cyclohexene; aromatic hydrocarbons: benzene, toluene, o-xylene, ethylbenzene) was repeatedly injected into a column, 2 m long and 0.4 cm I.D., packed with 25% squalane on Chromosorb W 60/80 mesh, over a period of 12 h. Hydrogen was used as the carrier gas at a flow rate of 40 ml/min. The column temperature and chart speed were 80° and 0.5 cm/min, respectively, and a single analysis took about 45 min. The retention distances, t_m, of peak maxima of the individual substances from the peak maximum of nonsorbed component (air) were measured by a rule graduated in millimeters. Mean values, $\bar{t}_m$, pertaining to the individual substances, were calculated. The calculated values of the standard deviations s_E may be looked upon, at the given number of measurements ($n = 15$), as a measure of precision of the absolute retention data, under the given conditions and experimental arrangements.

The values measured were analyzed by the usual statistical methods[15].

RESULTS AND DISCUSSION

Variance of absolute retention data

Absolute retention data are basic qualitative characteristics of the substances analyzed, from which various types of relative retention data are derived. Therefore, attention was given first to the variance of the absolute retention data of basic hydrocarbons which had been separated on squalane under conventional chromatographic conditions.
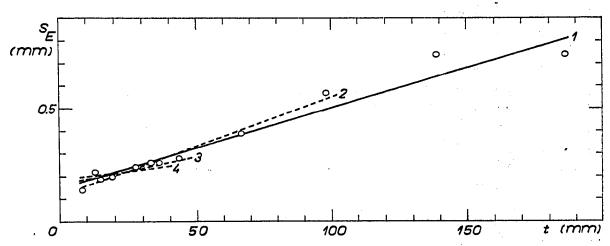

Fig. 1. The dependence of the standard deviations on the respective corrected absolute retention values of the substance analyzed. t is the corrected absolute retention value for the substance m (mm); s_E is the experimentally obtained estimation of the standard deviation of the value t (mm); the regression lines 1, 2, 3, and 4 have been calculated for 12, 10, 8, and 6 values of $[t, s_E]$, respectively.

It is evident from Fig. 1 that the magnitude of the absolute retention values of the substances analyzed is approximately linearly proportional to the respective standard deviation. Experimental data of the above type can be processed by the method of least squares[16], which simplifies further consideration or calculations with the measured data as well as the use of them. The linear regression of $[\bar{t}, s_E]$ values for twelve hydrocarbons gave the straight line

TABLE I.

VARIANCE OF THE ABSOLUTE RETENTION VALUE t OF THE SUBSTANCES ANALYZED

(a) $\bar{t}$ is the mean of the corrected retention values t_j (in mm) of the substance m; s is the estimation of the standard deviation of the corrected absolute retention value of the substance m; s_t has been calculated from the relation (1a); $s_E = \{[1/(n-1)] \sum_{j=1}^{n} (t_j - \bar{t})^2\}^{1/2}$, $n = 15$; v is the coefficient of variation of the corrected absolute retention values of the substance m, expressed as a percentage, $v_t = 100(s_t/t)$, $v_E = 100(s_E/t)$. (b) $\bar{t}_D$ is the mean of the measured lengths t_D corresponding to the corrected absolute retention value of the substance m; s_D is the standard deviation of the measured lengths t_D; s_D^2 is the variance of the measured lengths. (c) $\bar{t}_M$ is the mean of the measured lengths t_M corresponding to the corrected absolute retention value t_M of the substance m; s_M is the standard deviation of the measured lengths t_M; s_M^2 is the variance in determining the peak maximum, including the variance of the measured lengths, s_D^2. (d) F is the calculated value of the Snedecor criterion F; $F_1 = s_M^2/s_D^2$, $F_{crit} = 1.26$; $F_2 = s_m^2/s_D^2$, $F_{crit} = 1.74$; $F_3 = s_m^2/s_M^2$, $F_{crit} = 1.74$.

m	Ia						Ib			Ic			Id		
	$\bar{t}$ (mm)	s_E (mm)	v_E (%)	s_t (mm)	v_t (%)	s_t^2 (mm²)	$\bar{t}_D$ (mm)	s_D (mm)	$(t_D - \bar{t}_D)^2$ (mm²)	$\bar{t}_M$ (mm)	s_M (mm)	$(t_M - \bar{t}_M)^2$ (mm²)	F_1	F_2	F_3
1 n-Pentane	8.0	0.14	1.8	0.18	2.2	0.0324	8.4	0.10	0.15	8.2	0.05	0.04	3.12	9.81	3.15
2 Cyclopentene	12.8	0.22	1.7	0.19	1.5	0.0361	13.0	0.00	0.0	12.7	0.0	0.0		10.93	3.50
3 Cyclopentane	14.7	0.19	1.3	0.20	1.4	0.0400	15.0	0.00	0.0	14.9	0.13	0.24		12.12	3.88
4 n-Hexane	18.9	0.20	1.1	0.21	1.1	0.0441	19.0	0.00	0.0	19.0	0.11	0.18		13.36	4.28
5 Benzene	27.5	0.24	0.9	0.24	0.9	0.0576	28.0	0.00	0.0	27.5	0.08	0.08		17.43	5.59
6 Cyclohexane	33.8	0.26	0.8	0.27	0.8	0.0729	34.3	0.11	0.18	34.0	0.08	0.09		22.09	7.08
7 Cyclohexene	36.1	0.26	0.7	0.28	0.8	0.0784	36.6	0.10	0.13	36.3	0.08	0.09		23.76	7.61
8 n-Heptane	43.2	0.28	0.6	0.30	0.7	0.0900	43.3	0.05	0.04	43.0	0.05	0.04		27.27	8.74
9 Toluene	66.5	0.39	0.6	0.38	0.6	0.1444	66.7	0.00	0.0	66.5	0.05	0.04		43.75	14.02
10 n-Octane	98.1	0.57	0.6	0.50	0.5	0.2500	98.3	0.08	0.08	98.4	0.10	0.15		75.76	24.27
11 Ethylbenzene	138.8	0.72	0.5	0.64	0.5	0.4096	139.5	0.03	0.01	139.2	0.13	0.24		120.61	39.77
12 o-Xylene	186.0	0.72	0.4	0.81	0.4	0.6561	187.0	0.00	0.0	186.9	0.22	0.66		198.81	63.70
								$s_D^2 = 0.0033$			$s_M^2 = 0.0103$				

$$s = 0.15 + 0.0035t. \tag{1a}$$

If eqn. (1a) is rewritten in the form

$$s = a + bt \tag{1}$$

and rearranged to express the more illustrative coefficient of variation,

$$v = (a/t) + b \tag{1b}$$

it can be seen that the value of the coefficient of variation v decreases with rising value of t. With a sufficiently large t, the expression a/t would be expected to have no significant effect on the value of the coefficient of variation, and the latter will remain practically constant.

Thus, the value of the slope of regression line expressing the relation between absolute retention data and their estimated standard deviations represents the lowest value of the coefficient of variation of the absolute retention data measured under certain experimental conditions. If the regression line (eqn. 1) has been calculated from a sufficient number of points and if further measurements are carried out under identical conditions, then, after taking account of whether the chemical nature of the substance under analysis or some other factor might be the source of a substantial change in the variance of the retention data, it is possible to *calculate* from the relation (1a) the standard deviations of other substances, without carrying out the series of replicate injections usually necessary for calculating the standard deviation as a measure of the precision of the absolute retention values of the substance analyzed. If substances of a hydrocarbon nature are analyzed on a nonpolar phase, as has been the case in this work, the constants of the line (1) characterize, in a very useful way for the analyst, the instrument employed along with the experimental arrangements.

It is obvious that the linear regression of a different number of $[t, s_E]$ points produces straight lines with different a and b constants. Unless the values of the standard deviation of the absolute retention data of the other substances are determined by extrapolation, the variability of the constants a and b is practically insignificant, which is shown objectively in Fig. 1 which shows the regression lines obtained with 6, 8, 10, and 12 $[\bar{t}, s_E]$ points.

The measured t, s_E, v_E values as well as the calculated values of s_t, $s_t{}^2$, v_t pertaining to this part are quoted in Table Ia.

Analysis of the factors contributing to the resultant variance of absolute retention data

The value of $s_t{}^2$ represents the resultant variance of the absolute retention value t_m of the substance analyzed m. The effect of the individual factors which can, from the viewpoint of practical qualitative analysis, share in the ultimate value of the variance (*viz.* distance measurements, determination of the solute peak maxima, and the complex factor of the variability of working conditions) has been evaluated by analyzing the variance of experimental data in such a way that their variance is influenced by one particular factor at a time.

Analysis of the experimental data showed that the variance of the actual measurements of the retention distances was constant and independent of the absolute magnitude of the retention distance measured. The above variance, $s_D{}^2$, amounted to 0.0033 mm^2; this value was determined by repeated measurements ($n = 15$), in an identical chromatogram, of the lengths t_D corresponding to the distances of peak

axima of the individual substances from the peak of the nonsorbed substance, t_m. ie results of the individual measurements served for calculating the mean $\bar{t}_D$ and ie estimation of the standard deviation of the measurement of the respective length, $_,$. As there was no apparent dependence between the values of $\bar{t}_D$ and s_D (Table Ib), ie overall variance of measuring lengths, $s_D{}^2$, was calculated by

$$s_D{}^2 = \frac{1}{ij - 1} \sum_{i=1}^{1} \sum_{j=1}^{1} (t_{D_{ij}} - \bar{t}_{D_i})^2$$

The variance of the peak maximum determination for the substance under 1alysis was also constant and independent of the distance of peak maximum of the 1bstance from the peak maximum of the nonsorbed component. Its value was $_M{}^2 = 0.0103$, which was found by repeated determinations ($n = 15$), in an identical 1romatogram, of the peak maxima of the substances and the measured distances $_,$, corresponding to the replicate determinations of the peak maxima distances of 1e individual substances from the peak maximum of the nonsorbed component. The :sults of the measurements pertaining to each of the substances were processed to btain the mean $\bar{t}_M$ and the respective standard deviation s_M of determining the peak 1aximum. It follows from the way the determinations were made that the variance $_M{}^2$ also includes the variance of the measurements of length. There was no apparent ependence between the values of $\bar{t}_M$ and s_M (Table Ic); and the variance of the peak 1aximum, $s_M{}^2$, was calculated from:

$$s_M{}^2 = \frac{1}{ij - 1} \sum_{i=1}^{1} \sum_{j=1}^{1} (t_{M_{ij}} - \bar{t}_{M_i})^2$$

'he part of the resultant variance, $s_t{}^2$, of absolute retention data which was not .ccounted for by determining the peak maximum of the substance analyzed and by neasuring distances was held to be a measure of the variability of working conditions. :omparison of the variances $s_D{}^2$, $s_M{}^2$, and $s_t{}^2$ according to the Snedecor criterion Table Id) showed that the only significant source of the variability of absolute re- :ention data was a complex factor due to the variability of the working conditions. [t may be assumed, that the variance of the retention data due to this factor is charac- :eristic for the apparatus employed and for the conditions incident to the measure- ment.

A knowledge of the variances $s_D{}^2$, $s_M{}^2$, and $s_t{}^2$ is useful, from the practical point of view, in processing and evaluating the measured retention data for qualitative purposes, though it is evident that the conclusions based on comparing these variances depend on the actual values of the latter and, therefore, have no general applicability.

Variance of relative retention data

Relative retention data are easily available retention characteristics, and are very frequently used in gas chromatography practice. It has been assumed that the relating of the absolute retention data of substances analyzed to the retention datum of an appropriately chosen standard substance is sufficient to eliminate the effect of the variability of the basic working conditions, especially if the standard substance is present in every sample charge and if its retention time is close to that of the sub- stance analyzed[1,3]. Thus, relative retention data have a more general applicability

compared with absolute retention data, and qualitatively characterize the substance analyzed on a given stationary phase at a given temperature.

As stated above, absolute retention data have associated with them the standard deviation s, the dependence of which on the magnitude of the absolute retention data measured is described by eqn. 1. The relative retention value r_m of a substance m is the quotient of the corrected absolute retention values of the analyzed (m) and standard (z) substances, t_m and t_z, respectively. Therefore, the relative retention data have to be looked upon as a function of two variables, t_m and t_z, and its standard deviation can be calculated according to the general formula[15]

$$s_r = \frac{1}{t_z} \left(s_{t_m}^2 + \frac{s_{t_z}^2 t_m^2}{t_z^2} \right)^{1/2} \tag{2}$$

without carrying out replicate injections and calculations necessary to determine the standard deviation characterizing the precision of the relative retention data. This presumption was verified in the following way: The relative retention data for the hydrocarbons analyzed were calculated by relating the repeatedly measured absolute retention data of the individual hydrocarbons consecutively to n-pentane, n-hexane, n-heptane, and n-octane as internal standards. The fifteen values of the relative retention data of each of the hydrocarbons analyzed were processed to obtain the mean $\bar{r}_m$ and the respective estimations of variance, s_E^2. The subsequent calculation of the standard deviations of the relative retention data using eqn. 2 was carried out with the aid of the data concerning the variances s_m^2 and s_z^2 (the estimation of the standard deviations, s_E, for the relative retention data of the internal standards are obviously equal to zero; the estimations of standard deviation s_r for the standard substances have been calculated only formally for a case where $t_m \neq t_z$). The variances obtained experimentally are lower than the corresponding variances calculated by the derived relation (Table II).

The estimation of the standard deviations s_E and s_r illustrates the conception of the confidence interval ($\bar{r} - st$, $\bar{r} + st$) of relative retention data measured under certain experimental conditions (t denotes the Student coefficient in the interval of confidence).

Analysis of the factors contributing to the resultant variance of relative retention data.

The value s_r^2 represents the resultant variance of the relative retention data of the substances analyzed. In addition to the factors influencing the resultant variance of absolute retention data, there are further factors which may contribute to the value of the resultant variance. In connection with this concept, the effect of the *selection* of a particular internal standard has been studied as well as the possibility of calculating relative retention data by relating the absolute retention data to an internal standard which was not injected simultaneously with the mixture of substances analyzed.

It has been recommended that a hydrocarbon or a substance having a chemical nature similar to that of the substance analyzed which elutes approximately in the middle of the chromatogram of components to be separated[1,3] should be chosen as the internal standard. The standard deviation s_r of the relative retention data

BLE II

RIANCE OF THE RELATIVE RETENTION VALUE r OF THE SUBSTANCES ANALYZED

ɪ the mean of the relative retention values r_j of the substance m; s^2 is the variance of the relative ention values of the substance m; s_r^2 has been calculated by the relation (2); $s_E^2 = [1/(n-1)] \times$

ɪ $(r_j - \bar{r})^2$, $n = 15$; v is the coefficient of variation of the relative retention values of the sub- ʌnce m, expressed as a percentage $v_r = 100(s_r/r)$, $v_E = 100(s_E/r)$; the internal standard was ected simultaneously with the substance analyzed.

IIa					IIb				
Internal standard: n-pentane					Internal standard: n-hexane				
$\bar{r}$	$s_E^2 \times 10^4$	v_E (%)	$s_r^2 \times 10^4$	v_r (%)	$\bar{r}$	$s_E^2 \times 10^4$	v_E (%)	$s_r^2 \times 10^4$	v_r (%)
1.00	0	0	10.13	3.2	0.425	0.558	1.8	1.188	2.5
1.59	7.14	1.7	18.60	2.7	0.674	0.620	1.2	1.589	1.9
1.83	12.1	1.9	23.34	2.6	0.775	0.404	0.8	1.877	1.8
2.36	18.4	1.8	35.15	2.5	1.00	0	0	2.56	1.6
3.42	32.2	1.7	68.82	2.4	1.45	0.775	0.6	4.41	1.4
4.20	56.4	1.8	101.8	2.4	1.78	1.57	0.7	6.25	1.4
4.49	64.6	1.8	115.3	2.4	1.91	2.50	0.8	6.76	1.4
5.37	92.9	1.8	161.7	2.4	2.28	1.93	0.6	9.00	1.3
8.28	234.0	1.9	372.4	2.3	3.52	6.50	0.7	19.4	1.3
12.2	371.0	1.6	800.3	2.3	5.18	19.5	0.8	39.7	1.2
17.3	857.0	1.7	1588	2.3	7.33	37.4	0.8	77.4	1.2
23.1	1450	1.6	2894	2.3	9.82	78.7	0.9	136.9	1.2

an be expressed more illustratively in the form of the coefficient of variation,

$$v_r = (v_m^2 + v_z^2)^{1/2}, \tag{2a}$$

nd it can be seen from this that the coefficient of variation and, consequently, the tandard deviation of relative retention data will both be smaller when the values of he coefficients of variation of the substance analyzed and the internal standard ɪsed are low. Since the value of coefficient of variation decreases with an increase in he value of the absolute retention data (cf. eqn. 1b), one should choose, in calcu- ating relative retention data, from standards $z_1, z_2, \ldots, z_n$, for which $t_{z_1} < t_{z_2} < \ldots$ $< t_{z_n}$ and $v_{z_1} > v_{z_2} > \ldots > v_{z_n}$, a standard which elutes so far from the starting point ʜhat the coefficient of variation of the former can no longer significantly influence the esultant value of the coefficient of variation of the relative retention data. Indeed, ʜhe values of calculated (v_r) as well as experimentally obtained (v_E) coefficients of ɟariation decrease, in the series of the substances analyzed, with increasing reten- ʜion distance of the standard used from the start (Table II), in accordance with eqn. ₂a for calculating the coefficient of variation of relative retention data.

Hence, the resultant variance of the relative retention data is dependent on the selection of the internal standard and is lowest when the value of the coefficient ɔf variation of the internal standard used is lowest. However, the choice of the internal ʂtandard does not influence the qualitative values of the relative retention data, as ʜhe values of retention indices calculated from relative retention data expressed with different internal standards do not differ materially from each other (Table IVb).

Comparison, by means of the t test, of the mean values of the relative retention

c					IId				
Internal standard: n-heptane					Internal standard: n-octane				
	$s_E^2 \times 10^4$	v_E (%)	$s_r^2 \times 10^4$	v_r (%)	$\bar{r}$	$s_E^2 \times 10^4$	v_E (%)	$s_r^2 \times 10^4$	v_r (%)
186	0.12	1.9	0.19	2.3	0.082	0.0183	1.6	0.0361	2.3
296	0.14	1.3	0.24	1.7	0.130	0.0314	1.4	0.0441	1.6
340	0.11	1.0	0.27	1.6	0.150	0.0207	1.0	0.0484	1.5
438	0.07	0.6	0.32	1.3	0.193	0.0286	0.9	0.0529	1.2
636	0.13	0.6	0.49	1.1	0.280	0.0279	0.6	0.0784	1.0
782	0.04	0.3	0.67	1.1	0.345	0.0364	0.6	0.1089	0.9
836	0.06	0.3	0.76	1.1	0.368	0.0350	0.5	0.1156	0.9
00	0	0	0.96	1.0	0.441	0.0543	0.5	0.1444	0.9
54	0.21	0.3	1.96	0.9	0.679	0.193	0.7	0.2704	0.8
27	1.31	0.5	4.00	0.9	1.00	0	0	0.4900	0.7
21	3.23	0.6	7.29	0.9	1.42	0.785	0.6	1.00	0.7
31	5.93	0.6	12.3	0.8	1.90	0.500	0.4	1.69	0.6

data determined by using an internal standard injected simultaneously with the mixture of substances to be analyzed (Table IIc) with those determined by using an internal standard injected after every charge (Table IIIa), or after the fourth and eleventh charges of the mixture (Table IIIb), employing the mean value of the internal standard absolute retention data (Table IIIc), shows that the relative retention data of the hydrocarbons analyzed are not influenced significantly by any of the above methods of calculation. The measured values of the variances s_E^2 of the relative retention data expressed in the above way (Table III) do not amount to the values of the calculated variances s_r^2 (Table II). Hence, if the resultant variance of the absolute retention data has been tested and if the value of the respective standard deviation is satisfactory for the purposes of qualitative analysis, it is not absolutely necessary to inject the internal standard simultaneously with the mixture of analyzed substances in order to calculate the relative retention data.

Variance of retention indices

Retention indices[2] qualitatively characterize the analyzed substance on a given stationary phase at a given temperature. In calculating the retention index of a substance m,

$$I_{\text{temp.}}^{\text{st. ph.}}(m) = 100 \frac{\log x_m - \log x_{z'n}}{\log x_{z'n+1} - \log x_{z'n}} + 100n \qquad (3)$$

the retention parameter $x(x = V_g, t, r)$ of the substance analyzed is arranged so that it fits on the linear scale defined by the logarithms of the retention data of two

TABLE III

RELATIVE RETENTION DATA RELATED TO AN INTERNAL STANDARD NOT INJECTED SIMULTANEOUSLY
WITH THE MIXTURE OF SUBSTANCES ANALYZED

The meaning of $\bar{r}$ and s_E^2 is the same as in Table II; t is the Student coefficient calculated from the relation

$$t = (\bar{r}_A - \bar{r}_B) \Big/ \left[\frac{s_A^2 + s_B^2}{n-1}\right]^{1/2}, \ t_{crit.} = 2.05, \ n_A = n_B = 15;$$

the internal standard (n-heptane) was injected after every injection of the mixture (Table IIIa), after the fourth and eleventh injections of the mixture (Table IIIb), and, in the third case, the relative retention data have been calculated for the mean value $\bar{t}_x$ (Table IIIc).

m	IIIa			IIIb			IIIc		
	$\bar{r}$	$s_E^2 \times 10^4$	t	$\bar{r}$	$s_E^2 \times 10^4$	t	$\bar{r}$	$s_E^2 \times 10^4$	t
1	0.186	0.16	0	0.187	0.15	0.71	0.186	0.10	0
2	0.296	0.27	0	0.297	0.22	0.63	0.296	0.26	0
3	0.340	0.19	0	0.341	0.18	0.71	0.340	0.19	0
4	0.439	0.25	0.67	0.440	0.27	1.25	0.438	0.21	0
5	0.637	0.42	0.59	0.639	0.30	1.76	0.636	0.32	0
6	0.783	0.38	0.63	0.785	0.40	1.67	0.782	0.38	0
7	0.836	0.31	0	0.839	0.42	1.58	0.836	0.35	0
8	1.001	0.48	0.71	1.004	0.32	2.67	1.000	0.43	0
9	1.54	1.00	0	1.547	1.47	2.00	1.54	0.72	0
10	2.27	3.35	0	2.28	2.43	1.92	2.27	1.69	0
11	3.22	5.80	1.23	3.23	2.53	3.12	3.21	2.88	0
12	4.31	8.21	0	4.32	4.72	1.14	4.31	2.78	0

neighbouring n-alkanes z'_n and z'_{n+1}; with any temperature and stationary phase

$$I_{temp.}^{st. ph.} (n - C_n H_{2n+2}) = 100n \tag{3a}$$

Since retention data are measured with a standard deviation s_x under the given experimental conditions, the retention index has to be looked upon as a function of three variables, x_m, $x_{z'_n}$, and $x_{z'_{n+1}}$, so that its standard deviation is again characterized by the general formula[15]

$$s_{I_m} = \left[\frac{100 \log e}{\left(\log \frac{x_{z'_{n+1}}}{x_{z'_n}}\right)^2}\right] \left[\left(v_m \log \frac{x_{z'_{n+1}}}{x_{z'_n}}\right)^2 + \left(v_{z'_n} \log \frac{x_{z'_{n+1}}}{x_m}\right)^2 + \left(v_{z'_{n+1}} \log \frac{x_m}{x_{z'_n}}\right)^2\right]^{1/2}$$

$$\tag{4}$$

It is evident from the above relation that the variance of retention indices is not constant, but depends on the variance of the retention parameters from which it has been calculated. The value of the variance of the retention index is the lower if the values of the coefficients of variation of the individual variables are smaller. This premise has been verified empirically, and the respective results are quoted in Table IV. The experimentally obtained variances s_E^2 of the retention indices calculated from absolute retention data are lower or do not differ significantly from the variances s_I^2, obtained from the eqn. 4. The coefficients of variation v_E and v decrease with increasing distance of the substance analyzed from the start point. (The retention indices of substances $m = 1$–9 were calculated by interpolation between two closest alkanes and the retention indices of substances $m = 11, 12$ were calculated by extrapolation from the nearest pair of n-alkanes. The estimation of the variances s_E^2 of

TABLE IV

VARIANCE OF THE RETENTION INDICES OF THE SUBSTANCES ANALYZED

(a) I_t is the retention index of substance m calculated from the absolute retention value t; s^2 is the variance of the retention index of substance m; s_{It}^2 was calculated by eqn. 4; $s_E^2 = [1/(n-1)] \sum_{j=1}^{n} (I_{t_j} - I_t)^2$, $n = 15$; v is the percentage coefficient of variation of the retention index I_m of substance m, $v_{It} = 100(s_{It}/I_t)$, $v_E = 100(s_E/I_t)$. (b) I_r is the retention index of substance m calculated from the relative retention data; s is the standard deviation of the retention index I_r of substance m; s_{Ir} has been calculated by eqn. 4; the internal standard was injected simultaneously with the substances analyzed.

	IVa							IVb							
								n-Pentane[a]		n-Hexane[a]		n-Heptane[a]		n-Octane[a]	
m	I_t	s_E^2	s_E	v_E (%)	s_{It}^2	s_{It}	v_{It} (%)	I_r	s_{Ir}	I_r	s_{Ir}	I_r	s_{Ir}	I_r	s_{Ir}
1	500	0	0	0	13.9	3.7	0.7	500	5.2	500	4.2	500	3.9	500	3.8
2	554.0	1.48	1.2	0.2	4.95	2.2	0.4	554.0	3.9	553.9	2.8	554.2	2.4	553.8	2.4
3	570.3	1.16	1.1	0.2	3.98	2.0	0.4	570.4	3.9	570.2	2.6	570.4	2.2	570.5	2.1
4	600	0	0	0	3.62	1.9	0.3	600	3.8	600	2.5	600	2.2	600	2.0
5	645.2	0.37	0.6	0.1	1.81	1.3	0.2	645.1	3.6	645.1	2.2	645.2	1.7	645.0	1.5
6	670.2	0.14	0.4	0.06	1.46	1.2	0.2	670.1	3.7	670.0	2.1	670.2	1.6	670.3	1.4
7	678.2	0.17	0.4	0.06	1.44	1.2	0.2	678.2	3.7	678.3	2.1	678.3	1.6	678.1	1.4
8	700	0	0	0	1.26	1.1	0.2	700	3.6	700	2.0	700	1.5	700	1.3
9	752.7	0.34	0.6	0.08	0.76	0.9	0.1	752.7	3.5	752.9	1.9	752.7	1.4	752.7	1.2
10	800	0	0	0	0.62	0.8	0.1	800	3.5	800	1.8	800	1.3	800	1.2
11	842.2	0.89	0.9	0.1	0.48	0.7	0.1	842.5	3.4	842.3	1.8	842.3	1.3	842.8	1.1
12	878.1	0.65	0.8	0.1	0.43	0.7	0.1	877.8	3.5	877.9	1.9	878.2	1.2	878.4	1.0

[a] Internal standard.

TABLE V

RETENTION INDICES CALCULATED BY EXTRAPOLATION

I and s_E^2 have the same meaning as in Table IVa; t is the Student coefficient calculated by

$$ t = (\bar{I}_A - \bar{I}_B) \Big/ \left[\frac{s_A^2 + s_B^2}{n-1} \right]^{1/2}, \ t_{crit.} = 2.05, \ n_A = n_B = 15; $$

the retention indices have been calculated by extrapolation from the pairs: n-pentane and n-hexane (Table Va); n-hexane and n-heptane (Table Vb); and n-heptane and n-octane (Table Vc).

m	Va			Vb			Vc		
	I	s_E^2	t	I	s_E^2	t	I	s_E^2	t
1				496.2	5.66	5.98	494.9	9.39	6.24
2				552.2	2.12	3.55	551.3	3.40	4.57
3				569.1	1.11	2.99	568.4	2.56	3.68
4							599.4	0.82	2.48
5	643.6	1.85	4.01				644.9	0.74	1.06
6	667.7	2.75	5.51				670.0	0.16	1.37
7	675.4	3.79	5.26				678.1	0.17	0.64
8	696.4	4.88	6.09						
9	746.8	11.04	6.54	752.4	0.29	1.42			
10	792.1	20.86	6.48	799.4	0.78	2.54			

the retention indices of the n-alkanes used as a basis for the scale are obviously equal to zero; the values s_I^2 of the retention indices of the n-alkanes have been calculated from the values of $t z'_n$, $t z'_{n-1}$, and $t z'_{n+1}$.)

The values of standard deviation s_E and s_I illustrate the concept of the confidence interval ($I - st$, $I + st$) of the retention indices measured under certain experimental conditions.

Retention indices can be calculated from other data than absolute retention data in chromatographic practice. One often wants to compare different retention data for an identical substance, thus transforming the former into a universal scale of retention indices. A subsequent calculation of the retention indices (cf. eqn. 3) is simple when the retention data for all the necessary n-alkanes are known. If the precision of the retention data used in calculation is known, it is possible to obtain, employing eqn. 4, an idea of the precision of the additional retention indices calculated. If the different retention data of the substances analyzed have been obtained under really identical conditions, the retention indices calculated from them should agree well with each other.

Table IVb contains the retention indices of the hydrocarbons analyzed calculated from the relative retention data related, by turns, to n-pentane, n-hexane, n-heptane, and n-octane as internal standards. Their mean values are in good agreement with the mean retention indices calculated from the absolute retention data (see Table IVa). (The difference between the mean values of the retention indices calculated from absolute and relative retention data have been tested by the t-test. The calculated values of the Student coefficient were lower than the respective tabulated critical values.)

It is a different situation if the retention data of only some of the n-alkanes are available for calculating retention indices. In such cases, the retention indices of quite a variety of substances are calculated by extrapolation, often from a single pair

of neighbouring n-alkanes, or by interpolation between more distant n-alkanes. However, the linearity of the relation between the logarithms of the corrected retention data of the n-alkanes and their numbers of carbon atoms has not always been checked prior to the above calculation. The concepts published in the literature[17,18] on the linearity or nonlinearity of the above relation are different; it is obvious that retention indices suffer from bigger errors when calculated by extrapolation or by interpolation between more distant n-alkanes in limits where the relation between the logarithm of the retention data of the alkanes and their numbers of carbon atoms is nonlinear. Table V shows the retention indices of the hydrocarbons analyzed calculated by extrapolation from particular pairs of neighbouring n-alkanes (Table Va: n-pentane, n-hexane; Table Vb: n-hexane, n-heptane; Table Vc: n-heptane, n-octane) and the values of the calculated Student coefficients characterizing the significance of the difference between the values of the retention indices calculated by extrapolation and those obtained by interpolation between neighbouring n-alkanes (Table IVa).

CONCLUSIONS

The measurement of the absolute retention data has been carried out under conditions in which the data were not influenced by the amount of sample injected, sorption effects of the support, and different polarities of the solute and stationary phase. A linear relation, $s = a + bt$, has been found experimentally between the corrected absolute retention value t of the hydrocarbons analyzed and their standard deviation s_E, in which the constants a and b may be looked upon as characteristic for a given experimental arrangement and type of substance analyzed. From this viewpoint, relative retention data as well as retention indices of analyzed substances should be held as functions of variables characterized by certain variance. Their resultant variance can be found empirically (s_E^2); however, it can also be estimated (s_x^2) on the basis of the relation for calculating the standard deviation of a function. The calculated and empirically found variances of retention data agreed well with each other.

The fact that the retention value of the substance analyzed is characterized by a certain variance should not be neglected in processing measured data, determining and applying various types of correlation, and employing published retention data for the purposes of qualitative analysis; briefly, whenever the retention data are accepted and processed as a constant quantity. The author suggests that, in cases when it is expedient, the variance should be published along with the retention data for the experimental arrangement used, which would give some concept of the confidence interval for the published retention data.

REFERENCES

1 E. R. ADLARD, M. B. EVANS, A. G. BUTLIN, R. S. EVANS, R. HILL, J. F. K. HUBER, A. B. LITTLEWOOD, W. H. McCAMBLEY, J. F. SMITH, W. T. SWANTON AND P. A. T. SWOBODA, in A. GOLDUP (Editor), Gas Chromatographhy 1964, Elsevier, Amsterdam, 1965, p. 348.

2 E. KOVÁTS, in J. C. GIDDINGS AND R. A. KELLER (Editors), *Advances in Chromatography,* Vol. I, Marcel Dekker, New York, 1965, p. 229.
3 R. J. CVETANOVIC AND K. O. KUTSCHKE, in D. H. DESTY (Editor), *Vapour Phase Chromatography,* Butterworths, London, 1957, p. 87.
4 J. F. SMITH, *Chem. Ind. (London),* (1960) 1024.
5 D. AMBROSE, A. T. JAMES, A. I. M. KEULEMANS, E. KOVÁTS, H. RÖCK, C. ROUIT AND F. H. STROSS, in R. P. W. SCOTT (Editor), *Gas Chromatography 1960,* Butterworths, London, 1960, p. 423.
6 Data Subcommittee of GC Discussion Group of the Institute of Petroleum, *J. Gas Chromatog.,* 4 (1966) 1.
7 P. A. T. SWOBODA, in A. B. LITTLEWOOD (Editor) *Gas Chromatography 1966,* Elsevier, Amsterdam, 1967, p. 395.
8 J. C. LOEWENGUTH, *5th Intern. Symp. Separation Methods, Lausanne, 1969,* preprint, p. 56.
9 J. E. PURCELL AND E. W. MARCH, *Instr. News,* 19 (1968) 12.
10 W. EBING, *Chromatographia,* 2 (1969) 442.
11 J. E. OBERHOLTZER AND L. B. ROGERS, *Anal. Chem.,* 41 (1969) 1235.
12 A. W. C. VAN KEMENADE AND H. GROENENDIJK, *Chromatographia,* 2 (1969) 148.
13 C. A. CRAMERS, J. A. RIJKS, V. PACÁKOVÁ AND I. RIBEIRO DE ANDRADE, *J. Chromatog.,* 51 (1970) 13.
14 O. WIČAROVÁ, J. NOVÁK AND J. JANÁK, *J. Chromatog.,* 51 (1970) 3.
15 K. ECKSCHLAGER, *Chyby chemických rozborů,* SNTL, Prague, 1961.
16 K. ECKSCHLAGER, *Collection Czech. Chem. Commun.,* 25 (1960) 987.
17 H. GROENENDIJK AND A. W. C. VAN KEMENADE, *Chromatographia,* 1 (1968) 472.
18 L. RÖHRSCHNEIDER, *Chromatographia,* 2 (1969) 437.