

## GAS-LIQUID CHROMATOGRAPHY IN QUALITATIVE ANALYSIS

### PART II. THE REPRODUCIBILITY OF RETENTION DATA IN $R_{29}$ UNITS

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#### INTRODUCTION

The presentation of retention data in absolute units (specific retention volumes or partition coefficients), although conceptually simple, is beset with practical difficulties if reasonable accuracy is to be obtained<sup>1</sup>. For analytical purposes, retention ratios are generally used. However, owing to the wide range of column conditions and solutes concerned no single conventional standard material suffices. A wide range of arbitrary standards are therefore to be found in the literature.

This situation is unsatisfactory from a number of aspects. Thus, filing and tabulation of data become cumbersome, comparison of data using different standards is difficult and thermodynamic relationships are indeterminate.

A note has been published<sup>2</sup> by one of the present authors which shows that retention ratios relative to *n*-nonane can be obtained by extrapolation or interpolation of the close linear relation between log (retention) and carbon number in the homologous *n*-paraffin series. This is referred to as the "log plot" in this paper. The reasons for the particular choice of *n*-nonane as standard are given later.

The precision of the method clearly depends on the precision with which the retentions of the *n*-paraffins, or any other internal standards, can be expressed relative to the retention of *n*-nonane which in turn depends on the accuracy of the interpolation or extrapolation of the linear "log plot". This is discussed in some detail in the theoretical section.

#### EXPERIMENTAL

##### *Apparatus*

Only conventional and simple gas chromatography equipment was used throughout this investigation. Columns were 5 feet  $\times$  4 mm in pyrex glass using a modified flame ionisation detector<sup>3</sup>. The carrier gas was a 3:1 (by volume) mixture of hydrogen and nitrogen. The carrier gas flow rate and column inlet pressure were controlled only by an OR12 pressure regulator (British Oxygen Gases Ltd.). The column temperature was regulated by a vapour jacket consisting of boiling methanol (64.7°), water (100.0°) or *n*-pentanol (138.0°). These boiling points were subject to some variation

due to changes in barometric pressure which ranged from 740 to 765 torr. The stationary phases were supported on acid-washed (1% phosphoric acid) 60–72 mesh celite (J.J.'s, Ewell, Surrey, England) and in certain cases on 60–72 mesh C.22 firebrick (J.J.'s).

In this investigation the only stationary phases used were dinonyl phthalate (BDH for chromatography) and squalane (BDH for chromatography). In our experience, polyethylene glycol and silicone columns, although otherwise useful, change their character on usage even at quite moderate temperatures. Samples (approximately 10  $\gamma$ ) for analysis were introduced by means of stainless steel capillary pipettes<sup>4</sup>. Columns were operated at or near optimum carrier gas flow rate (approx. 50 c.c./min, measured at room temperature and at column outlet pressure) and had efficiencies between 400 and 500 theoretical plates per foot of column.

### *Materials*

Solutes were selected with a view to having as wide a range of solvent interactions as possible. Thus in respect of dipole moments these ranged from zero for the *n*-paraffins to about 4.0 Debye units for aromatic nitro compounds. Group polarisabilities ranged from 1.68 for the C—H bond of *n*-paraffins to 8.1 for the S—S bond in disulphides (polarisability expressed as contributions to the Lorentz molar refraction). Hydrogen bond acceptor and donor properties ranged from zero in the *n*-paraffins to the strong tendency in both respects for alcohols.

The solutes studied were all commercially available and were either predominantly pure (checked by infra-red analysis and gas-liquid chromatography where appropriate) or had a component which was clearly identifiable (*e.g.* from a homologous series gas-liquid chromatography plot).

### *Calculation procedure for $R_{x_0}$ values*

A convenient calibration series of *n*-paraffins was injected into the column and a chromatogram obtained. Since the overall reproducibility was being investigated this was repeated for each  $R_{x_0}$  value determined. In normal practice this calibration chromatogram needs only to be repeated at infrequent intervals to check any change of column characteristics with usage.

Retention *distances* on the chart were then measured from the "column dead volume point" to the peak maxima.

With detectors insensitive to air no "air peak" is observed and this presents some difficulty. This was obviated by the following procedure. A calculated value for the column interstitial volume was first taken, converted into chart distance, and subtracted from retention distances measured from the point of injection. These adjusted retention distances were then plotted against carbon number (on 11½ in.  $\times$  16½ in. 2 or 3 cycle log-linear graph paper). If in fact the calculated interstitial volume was in error some curvature of the plot at low retention times was apparent. The further correction required could, however, easily be computed from the expression

$$\delta a = \frac{2.303 \sum [R(\delta \log R)^2]}{\sum [\delta \log R]}$$

where  $R$  = retention distance from assumed dead volume point on chart

$\delta \log R = \log R_{\text{observed}} - \log R_{\text{extrapolated}}$

$R_{\text{extrapolated}}$  = value obtained from extrapolation of linear part of graph

$\delta a$  = further correction to retention distance to be subtracted from the first set of adjusted retention distances.

Provided the first calculated interstitial volume of the column was not seriously in error the above correction gave very precise values for adjusted retention distances and on replotting the data no curvature was apparent.

From the corrected graph the retention distance for  $n$ -nonane was obtained by interpolation or extrapolation. Within the temperature range studied this could be done graphically with sufficient accuracy. At very much higher temperatures "least squares" methods *may* be required.

From the value for the adjusted retention distance of  $n$ -nonane obtained from the corrected graph, the relative retentions of the  $n$ -paraffins within the calibration with respect to  $n$ -nonane were calculated from the relation

$$R_{N_9} = R_N/R_9$$

where  $R_{N_9}$  = retention of paraffin relative to  $n$ -nonane

$R_N$  = adjusted retention distance on chart of the paraffin

$R_9$  = adjusted retention distance on chart of  $n$ -nonane deduced from the graph.

On occasion the use of  $n$ -paraffins as internal standards is inconvenient. In this case a suitable internal standard was added to the calibration series and the retention of the proposed internal standard relative to  $n$ -nonane thus obtained. On very polar stationary phases the concentrations of  $n$ -paraffins in this calibration generally need to be small in order to obviate band broadening due to delayed volatilisation. With the high sensitivity detectors now available this presents little difficulty.

Having thus obtained the retentions of the  $n$ -paraffins or other standards relative to  $n$ -nonane one or more of these were used as conventional internal standards to measure the retentions of various solutes relative to  $n$ -nonane from the relation

$$R_{x_9} = R_{xN} \times R_{N_9}$$

where  $R_{x_9}$  = retention of material being measured relative to  $n$ -nonane

$R_{xN}$  = retention of material being measured relative to the internal standard

$R_{N_9}$  = retention of standard relative to  $n$ -nonane.

For reasons discussed in the theoretical section, wherever possible, two internal standards eluting either side of the material being measured, were used and the mean  $R_{x_9}$  value taken.

For calibration purposes only  $n$ -paraffins above  $n$ -butane should be used since on theoretical grounds the linearity of the "log plot" should deteriorate below  $n$ -butane. This is not generally of concern since these low molecular weight paraffins are more conveniently analysed by gas-solid chromatography.

*Temperature cycling of columns*

In order to simulate normal practice in analytical laboratories the columns were temperature cycled as follows: (1) Dinonyl phthalate columns, 65° (7 h), 100° (7 h), 65° (7 h), 100° (7 h) etc. (2) Squalane columns, 65° (7 h), 100° (7 h), 138° (7 h), 65° (7 h) etc.

In the lower temperature range it is possible that the reproducibility of retention data would be better than that observed if the column was not operated at higher temperatures.

## THEORETICAL

*The reproducibility of conventional retention ratios*

The simplest case to consider is where the standard and the unknown are run on separate chromatograms. If it is assumed that the percentage standard deviation of a retention time is independent of retention time (this has experimental support provided rapidly eluted materials are excluded) then the theory of random error propagation predicts that

$$\varepsilon_{xN} = (\varepsilon_x^2 + \varepsilon_N^2)^{\frac{1}{2}} = 2^{\frac{1}{2}} \varepsilon_0 \quad (1)$$

where  $\varepsilon_{xN}$  = % standard deviation in the retention ratio

$\varepsilon_x = \varepsilon_N = \varepsilon_0$  = % standard deviation in retention time.

In practice, however, the standard and the unknown are almost always run together on the same chromatogram (Internal standard technique). The theoretical discussion of the standard deviation of the retention ratio under these conditions is complicated except for the unique cases (1) where the errors in operating conditions are subject to rapid random fluctuations or (2) where the operating conditions are subject to systematic linear drift. Under the latter conditions and taking the special case where errors are due to a linear drift in carrier gas flow rate then

$$\varepsilon'_{xN} = \frac{100\alpha}{2F_0} \left| t_2 - t_1 \right| \quad (2)$$

where  $F = F_0 + \alpha t$  = carrier gas flow rate at time  $t$

$F_0$  = flow rate at the time of elution of the "air peak"

$t_1$  = elution time of standard (measured from air peak)

$t_2$  = elution time of unknown (measured from air peak).

Clearly the error will be zero when  $t_2 = t_1$ , and hence the desirability of using an internal standard with a similar retention time to the unknown. Exact equality is not practicable since the peak must be resolved and an infinite range of standards would be required.

If two internal standards can be used which have a precise known relation to some primary standard then the percentage error of the mean retention ratio relative to this primary standard under conditions of linearly drifting carrier gas flow rate will be given by

$$\varepsilon'_{xp} = \frac{100\alpha}{2F_0} \left| t_2 - \frac{t_3 + t_1}{2} \right| \quad (3)$$

where  $t_1$  = retention time of first standard

$t_2$  = retention time of unknown

$t_3$  = retention time of second standard

and,  $t_1 < t_2 < t_3$ .

If therefore the retention of the unknown is the mean of the retentions of the two internal standards, errors due to a linearly drifting carrier gas flow rate will be eliminated. This is in principle a practical proposition since all peaks can be resolved under these conditions. Moreover although exact equality of  $t_2$  and  $(t_3 + t_1)/2$  can rarely be obtained, approximate equality pertains when the unknown is eluted between the two internal standards. This is a readily obtainable condition with the  $R_{x_9}$  method using two convenient  $n$ -paraffins as internal standards.

Similar but more complex considerations apply to small linear drifts in other operating conditions (temperature, amount of stationary phase in column) provided the *character* of the stationary phase is not drifting. When the drift is no longer linear with time the errors can be greater than those suggested by eqns. (2) and (3).

The following general principles, however, still apply namely: (1) Retention ratios measured on a single chromatogram should be more reproducible than those derived from separate chromatograms. (2) The accuracy with the former method should improve as the retentions of the internal standard and the unknown approach each other. (3) A method which involves the use of two internal standards more or less symmetrically disposed either side of the unknown should be more reproducible than methods using a single standard similarly disposed, provided the relations between the internal standards and the primary standard are precisely known.

*The reproducibility of retention ratios in theoretical nonane units ( $R_{x_9}$ )*

Since  $R_{x_9} = R_{xN} \times R_{N_9}$  then by the law of random error propagation

$$\epsilon_{x_9}^2 = \epsilon_{xN}^2 + \epsilon_{N_9}^2 \quad (4)$$

This applies when the two determinations are on separate chromatograms.  $\epsilon_{xN}$  is the % standard deviation for a conventional retention ratio. The critical factor therefore which will determine the % standard deviation of retention ratios in  $R_{x_9}$  units is the quantity  $\epsilon_{N_9}$  which is the percentage standard deviation for the retention of the standard relative to  $n$ -nonane. This itself is a composite entity since

$$R_{N_9} = R_N/R_9$$

where  $R_N$  = retention of standard

$R_9$  = retention of  $n$ -nonane.

Under the worst and never used conditions where these retentions are measured on separate chromatograms

$$\epsilon_{N_9}^2 = \epsilon_N^2 + \epsilon_9^2 \quad (5)$$

Although these conditions never apply in practice it is nevertheless important to evaluate the probable value of  $\epsilon_9$ , the % standard deviation for the retention of  $n$ -nonane using separate chromatograms throughout.

The value of  $R_9$  as stated previously is obtained by extrapolating the best straight line of the  $\log R_N$  vs. carbon number plot of the  $n$ -paraffins. The probable error for  $R_9$  in such a calibration is given by

$$\varphi_9^2 = \frac{\sum (i - 9)^2}{v \sum i^2 - (\sum i)^2} \quad (6)$$

where  $\varphi_9$  = % probable error of  $R_9$  / % probable error of a single retention time

$i$  = the carbon number of each paraffin in the calibration

$v$  = the number of paraffins in the calibration.

This equation applies to the worst possible system of calibration where each paraffin is run on a separate chromatogram. Under normal calibration conditions of a single chromatogram for the whole series,  $\varphi_9$  may be expected to be much smaller.

The value of  $\varphi_9$  is clearly a function of the calibration range of  $n$ -paraffins. If all the  $n$ -paraffins with carbon numbers between  $m$  and  $n$  are used in the calibration eqn. (6) reduces to

$$\varphi_9^2 = \frac{2 \{ 54 (9 - n - m) + 2 (n^2 + m^2 + mn) + n - m \}}{(n - m) (n - m + 1) (n - m + 2)} \quad (7)$$

or in more general terms

$$\varphi_{\mathcal{N}}^2 = \frac{2 \{ 6\mathcal{N} (\mathcal{N} - n - m) + 2 (n^2 + m^2 + mn) + n - m \}}{(n - m) (n - m + 1) (n - m + 2)} \quad (8)$$

where  $\mathcal{N}$  is the carbon number of the  $n$ -paraffin primary standard.

By using eqn. (7) it is possible to evaluate the minimum value of  $n$  for each value of  $m$  which will make  $\varphi_9 \leq 1$ .

The minimum calibration ranges shown in Table I have been selected on this basis.

In practice using a single chromatogram for the whole calibration these ranges are more than sufficiently wide and the accuracy correspondingly greater. By partially differentiating eqn. (8) with respect to  $\mathcal{N}$ ,  $\varphi_{\mathcal{N}}^2$  can be shown to have a minimum value when  $\mathcal{N} = (n + m)/2$ . That is to say the best primary standard from this point of view is one near the middle of the range of  $n$ -paraffins which are amenable to GLC.

Although  $n$ -nonane is somewhat below the middle of the range of  $n$ -paraffins which are amenable to GLC, primary standard paraffins with a higher carbon number would make calibration times at the lower end of the scale impossibly long.  $n$ -Nonane would therefore undoubtedly appear to be the best standard from a practical point of view.

The maximum practicable calibration range of  $n$ -paraffins is determined by the maximum convenient elution time for the last peak ( $R_n$ ). Now owing to the linearity of the log plot

$$R_n = R_m 10^{b(n - m)} \quad (9)$$

where  $R_m$  = retention time of first peak (measured from air peak)

$b$  = slope of log plot.

TABLE I  
MINIMUM AND MAXIMUM CALIBRATION RANGES, SELECTED FOR  
SMALLEST NUMBER OF PEAK MEASUREMENTS\*

<i>m</i>	<i>n</i>	<i>n max</i> **	Column temp. °C	No. of peak measurements	No. of determinations	Minimum running time, min
1	8		—86	8	1	1.8 · 10 <sup>7</sup> ***
2	8		—64	7	1	2.4 · 10 <sup>5</sup> ***
3	8		—42	6	1	8.6 · 10 <sup>3</sup> ***
4	7	8	—20	12	3	470
5	9	9	0	5	1	330
6	9	11	24	4	1	55
7	9	12	46	3	1	14
8	9	14	68	2	1	5
9	10	16	90	2	1	4
10	15	18	112	6	1	50
11	18	21	135	8	1	97
12	19	23	156	8	1	59
13	22	26	179	10	1	85
14	24	29	200	11	1	68
15	26	33	223	12	1	53
16	28	37	246	13	1	42
17	30	45	267	14	1	25

\* Columns 3, 4 and 7 in this Table are purely illustrative and refer to a 5% silicone 704 column of the type described under EXPERIMENTAL.

\*\* *n max* is the highest value of *n* for the above column for which the time for a chromatogram does not exceed 8 h.

\*\*\* These values are impossibly long.

The minimum practical value of  $R_m$  is that which can be accurately measured on the chart. With conventional potentiometric recorders having a maximum chart speed of 1 in. per min this minimum accurately measurable elution time is probably 1–2 min.

The factor *b* depends critically on the nature of the stationary phase and the temperature. Representative values for *b* are shown in Table II.

The maximum calibration ranges shown in Table I are for a 5% silicone MS704 column (4 mm × 5 feet) at temperatures appropriate to a retention time of about

TABLE II  
EXPERIMENTAL VALUES OF *b* FOR *n*-PARAFFINS IN THE EQUATION  $\log R = a + bN$

	20°	65°	100°	138°	160°
10 <sup>3</sup> × 1/ <i>T</i>	3.413	2.959	2.681	2.433	2.309
PEG*	0.380	0.301	0.230	0.204	
DNP	0.477	0.377	0.311	0.258	
Sil. 704*	0.477	0.362	0.301	0.146	0.114
Squalane	0.492	0.391	0.324	0.264	

\* These values are very approximate due to column instability.

1 min for the first member of the calibration series and about 8 h for the last member at a carrier gas flow rate of approximately 50 c.c./min.

This type of stationary phase has high  $b$  values for  $n$ -paraffins and represents difficult conditions in respect of time required for calibration.

In spite of this the minimum calibration ranges required for accuracy are well within the maximum convenient calibration ranges.

Under other conditions (polar stationary phases, normal capillary columns) calibration times are much reduced.

Summarising, under the worst conditions of separate chromatograms throughout and using a single secondary standard

$$\epsilon_{xN} = \sqrt{2} \epsilon_0 \quad (10)$$

$$\epsilon_{x9} = 2\epsilon_0 \quad (11)$$

That is to say the probable error for retention data in  $R_{x9}$  units should be  $\sqrt{2}$  times the probable error in the conventional method.

However, the facts that the whole calibration is on a single chromatogram and that two internal  $n$ -paraffin standards with the unknown can be used in determining  $R_{x9}$  makes the probable error in  $R_{x9}$  considerably less.

In many cases this improvement is particularly marked for retention data in  $R_{x9}$  units and in consequence data in these units are rarely inferior in reproducibility compared with conventional methods and frequently superior (see RESULTS).

#### *The relation between $R_{x9}$ and the retention index*

KOVATS<sup>5</sup> has defined the retention index as

$$I = 200 \frac{\log R(x) - \log R(P_z)}{\log R(P_{z+2}) - \log R(P_z)} + 100Z \quad (12)$$

where  $R(x)$  = retention of unknown

$R(P_z)$  = retention of  $n$ -paraffin with  $z$  carbon atoms

$R(P_{z+2})$  = retention of  $n$ -paraffin with  $z + 2$  carbon atoms.

KOVATS does not comment on the physical significance of  $I$ . It is in fact  $100 \times$  the carbon number of a hypothetical  $n$ -paraffin having the same retention as the unknown. In the following discussion we replace  $I$  by  $I'$  where  $I' = I/100$  since in our view  $I'$  is a more convenient parameter.

$R_{x9}$  and  $I'$  are simply related by the expression,

$$I' = \frac{\log R_{x9}}{b} + 9 \quad (13)$$

The recommended experimental procedures for obtaining  $R_{x9}$  and  $I'$  are somewhat different, however. Thus the  $R_{x9}$  method suggested in this paper uses a number of  $n$ -paraffins in the calibration and therefore incorporates a check on the accuracy



of the column interstitial volume apart from a generally higher standard of reproducibility which is shown in the experimental results.

The  $R_{x_9}$  method is in our view more convenient for analytical purposes in that the  $R_{x_9}$  value is directly proportional to elution time. In the retention index system relative elution times can only be calculated *via* a knowledge of the  $b$  factor for the column.

The retention index is reputed to have a virtually zero temperature coefficient<sup>5</sup>, but since

$$\frac{d I'}{d(1/T)} = \frac{\partial I'}{\partial \ln R_{x_9}} \frac{d \ln R_{x_9}}{d(1/T)} + \frac{\partial I'}{\partial b'} \frac{d b'}{d(1/T)} \quad (14)$$

where  $T$  = absolute temperature and  $b' = 2.303b$ . Then from eqn. (13)

$$\frac{d I'}{d(1/T)} = \frac{1}{b'} \frac{d \ln R_{x_9}}{d(1/T)} - \frac{\ln R_{x_9}}{b'^2} \frac{d b'}{d(1/T)} \quad (15)$$

$$= \frac{1}{b} \frac{d \log R_{x_9}}{d(1/T)} - \frac{\log R_{x_9}}{b^2} \frac{d b}{d(1/T)} \quad (16)$$

The condition for zero temperature coefficient for  $I'$  is thus

$$b \frac{d \log R_{x_9}}{d(1/T)} = \log R_{x_9} \frac{d b}{d(1/T)} \quad (17)$$

When this condition does not hold, the temperature coefficient of  $I'$  will clearly be a function of temperature.

#### *The thermodynamic significance of $R_{x_9}$ values*

Most work of a thermodynamic nature in gas chromatography has followed thermodynamic convention for vapour-solution equilibria and taken the pure liquid solute at the temperature and average pressure of the column as the standard state<sup>6,7</sup>.

This introduces a number of experimental complications in that to determine true activity coefficients at infinite dilution ( $\gamma^\circ$ ) the fugacities of the pure solutes must also be determined. In general this means determining the vapour pressure of the pure solutes and correcting for non-ideality of the vapours via knowledge of the molar volumes in the liquid phase and the second virial coefficients. For most compounds this data is not available. If, however, the vapour at infinite dilution is taken as the standard state it is possible to calculate from  $R_{x_9}$  values (provided small samples are used) the partial molar free energy of solution increment over that for  $n$ -nonane at infinite dilution by the simple relation

$$\overline{\Delta G}_{x_9} = \underline{RT} \ln R_{x_9}$$

moreover

$$\frac{d \ln R_{x_9}}{d(1/T)} = \frac{\overline{\Delta H}_{x_9}}{\underline{R}}$$

and

$$\frac{1}{T} \frac{d \ln R_{x_9}}{d(1/T)} - \ln R_{x_9} = \frac{\overline{\Delta S}_{x_9}}{\underline{R}}$$

where  $\overline{\Delta G}_{x_9}$  = increment in partial molar free energy of solution from the gas phase at infinite dilution compared with *n*-nonane

$T$  = absolute temperature

$\overline{\Delta H}_{x_9}$  = increment in partial molar enthalpy of solution at infinite dilution from the gas phase compared with *n*-nonane

$\overline{\Delta S}_{x_9}$  = associated entropy term

$R$  = gas constant.

$\overline{\Delta H}_{x_9}$  is in general sufficiently invariant with  $1/T$  to make possible good temperature interpolations for  $R_{x_9}$ , by plotting  $\log R_{x_9}$  against  $1/T$ .

The use of  $\overline{\Delta G}_{x_9}$ ,  $\overline{\Delta H}_{x_9}$  and possibly  $\overline{\Delta S}_{x_9}$  would appear justified by the fact that such terms are generally an additive function of chemical group contributions<sup>8-10</sup>.

### RESULTS

$R_{x_9}$  values for various solutes on squalane and dinonyl phthalate at various temperatures are shown in Tables V-IX. Alongside the  $R_{x_9}$  value the standard deviation and the percentage standard deviation (obtained from at least nine determinations of  $R_{x_9}$ ) are quoted. The remaining columns in Tables V-IX give the conventional retention ratios taking various *n*-paraffins as internal standards. The standard deviations and percentage standard deviations of these values are also quoted.

$R_{x_9}$  values for *n*-paraffins are shown in Tables III-IV together with percentage standard deviations.

TABLE III  
n-PARAFFINS ON 20% DNP/CELITE

Substance	64.7° $b = 0.377 Pb = 0.0009$			100° $b = 0.311 Pb = 0.0009$		
	$R_{x_9}$	$P_{x_9} \cdot 10^3$	$\sigma_{x_9}$	$R_{x_9}$	$P_{x_9} \cdot 10^3$	$\sigma_{x_9}$
<i>n</i> -Pentane	0.031	0.79	2.5			
<i>n</i> -Hexane	0.074	0.79	1.1	0.113	2.0	1.8
<i>n</i> -Heptane	0.176	0.79	0.45	0.237	2.6	1.1
<i>n</i> -Octane	0.421	1.0	0.24	0.487	2.5	0.51
<i>n</i> -Nonane	1.000	0.00	0.00	1.000	0.00	0.00
<i>n</i> -Decane	2.377	4.5	0.19	2.051	6.0	0.29
<i>n</i> -Undecane	5.657	15.4	0.27	4.196	16.0	0.38
<i>n</i> -Dodecane				8.556	43	0.50
<i>n</i> -Tridecane				17.52	131	0.75
	$K$ <i>n</i> -nonane $\sim 0.76 \cdot 10^3$			$K$ <i>n</i> -nonane $\sim 0.22 \cdot 10^3$		

It is to be noted that although the standard deviation is always low, the values vary widely. In the case of rapidly eluted materials a relatively high standard deviation is to be expected but, apart from this factor, the origin of the diversity of standard deviations is hard to explain.

The theory of the  $R_{x_9}$  method suggests that owing to the fact that at elevated temperatures calibration ranges of *n*-paraffins were used which are wider than neces-

TABLE IV  
*n*-PARAFFINS ON 20% SQUALANE/CELITE

Substance	64.7° $b = 0.391 Pb = 0.0007$			100° $b = 0.324 Pb = 0.0016$			138° $b = 0.264 Pb = 0.0007$		
	$R_{x_0}$	$P_{x_0} \cdot 10^3$	$\sigma_{x_0}$	$R_{x_0}$	$P_{x_0} \cdot 10^3$	$\sigma_{x_0}$	$R_{x_0}$	$P_{x_0} \cdot 10^3$	$\sigma_{x_0}$
<i>n</i> -Pentane	0.027	0.73	2.7	0.050	1.3	6.6			
<i>n</i> -Hexane	0.067	0.75	1.1	0.105	1.3	1.3			
<i>n</i> -Heptane	0.165	0.84	0.51	0.224	1.8	0.79	0.305	3.3	1.1
<i>n</i> -Octane	0.407	1.5	0.37	0.474	2.6	0.54	0.549	3.7	0.68
<i>n</i> -Nonane	1.000	0.0	0.00	1.000	0.00	0.00	1.000	0.00	0.00
<i>n</i> -Decane	2.459	4.6	0.19	2.110	7.2	0.34	1.839	4.8	0.26
<i>n</i> -Undecane	—	—	—	4.446	39	0.89	3.386	8.2	0.24
<i>n</i> -Dodecane	—	—	—	9.410	77	0.82	6.230	20	0.33
<i>n</i> -Tridecane	—	—	—	—	—	—	11.48	49	0.43
<i>n</i> -Tetradecane	—	—	—	—	—	—	21.22	120	0.58

$K$  *n*-nonane  $\sim 1.2 \cdot 10^3$    
  $K$  *n*-nonane  $\sim 0.31 \cdot 10^3$    
  $K$  *n*-nonane  $\sim 0.13 \cdot 10^3$

sary to maintain constant reproducibility of  $R_{x_0}$ , then the reproducibility should improve with increase in temperature. By taking the mean standard deviation at each temperature, for all the materials studied in this paper, this trend is evident (Table X). Also in this table are shown (1) the mean standard deviation of retention ratios using a single *n*-paraffin standard with a retention in the middle range and (2) the mean standard deviation of retention ratios using in each case as internal standard the *n*-paraffin with the closest retention to that of each substance.

In general terms the reproducibility of the  $R_{x_0}$  method compares with (1) but slightly inferior to (2) as predicted on theoretical grounds.

One concludes that provided identical column characteristics pertain,  $R_{x_0}$  values are generally reliable to within the limits set by a standard deviation of approximately 0.5%.

Reproducibility between laboratories would therefore appear to rest largely on the identity of column characteristics. Methods by which column characteristics can be defined are being studied in these laboratories and will be published in due course.

The importance of column characterisation cannot be overemphasised, since variations in the purity or composition of the stationary phase (either initially or due to degradation with usage), the nature of the support and the surface area/volume ratio for the stationary phase may all substantially affect column characteristics in certain respects. This is shown in Tables XI–XII.

The onset of column degradation can be very sudden in certain cases. Thus a squalane column maintained at 100° gave reproducible  $R_{x_0}$  values for about 140 h. Beyond this time although the  $R_{x_0}$  values for relatively non polar materials showed little change, the  $R_{x_0}$  values for polar or polarisable materials showed a marked drift (Table XIII).

TABLE V  
20% DNP/CELITE 64.7°  $b = 0.377$   $K$   $n$ -NONANE  $\sim 0.76 \cdot 10^3$

Substance	$R_{x_3}$ method			$n$ -Octane = 1.00			$n$ -Nonane = 1.00			$n$ -Decane = 1.00		
	$R_{x_3}$	$P_{x_3} \cdot 10^3$	$\sigma_{x_3}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$
Methyl ethyl ketone	0.163	1.8	1.1	—	—	—	0.164	1.9	1.1	0.069	1.0	1.5
Butan-1-ol	0.417	4.4	1.1	—	—	—	0.418	4.1	0.97	0.176	3.0	1.7
Oct-1-ene	0.426	0.90	0.21	—	—	—	0.426	1.2	0.29	0.179	0.71	0.40
$n$ -Butyl acetate	0.786	3.3	0.41	1.867	6.5	0.35	0.785	4.6	0.58	—	—	—
Chlorobenzene	1.267	2.6	0.20	—	—	—	1.268	1.6	0.13	0.533	2.2	0.92
Hexane-1-thiol	1.863	4.1	0.22	—	—	—	1.864	4.1	0.22	0.785	3.24	0.41
Isopropylbenzene	1.961	2.19	0.11	4.664	9.9	0.21	1.959	6.2	0.31	—	—	—
Phenyl methyl ether	2.594	5.0	0.19	6.159	24	0.39	2.590	8.9	0.34	—	—	—

TABLE VI  
20% DNP/CELITE 100°  $b = 0.311$   $K$   $n$ -NONANE  $\sim 0.22 \cdot 10^3$

Substance	$R_{x_3}$ method			$n$ -Nonane = 1.00			$n$ -Decane = 1.00			$n$ -Undecane = 1.00		
	$R_{x_3}$	$P_{x_3} \cdot 10^3$	$\sigma_{x_3}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$
Butan-1-ol	0.438	3.3	0.76	0.438	3.4	0.77	0.214	2.1	0.98	0.104	1.6	1.5
Ethylbenzene	1.243	4.6	0.37	1.243	2.5	0.20	0.606	2.1	0.34	0.294	2.7	0.91
Hexan-1-ol	1.952	5.0	0.26	1.949	5.9	0.30	—	—	—	0.465	2.1	0.44
2,6-Dimethyl-octa-2,6-diene	2.323	8.9	0.38	2.323	7.5	0.32	—	—	—	0.555	1.5	0.26
$n$ -Butyl $n$ -butyrate	2.804	8.6	0.31	2.800	5.7	0.21	—	—	—	0.669	1.0	0.15
Di-isopropyl disulphide	3.601	6.9	0.19	3.597	8.9	0.25	—	—	—	0.859	2.3	0.27
Ethyl phenyl ether	3.712	5.3	0.14	3.712	11	0.29	1.811	3.3	0.18	0.884	2.1	0.24
Octan-1-ol	8.400	19	0.22	8.390	24	0.28	—	—	—	2.003	3.6	0.18

TABLE VII  
20% SQUALANE/CELITE 64.7°  $b = 0.391$   $K$  *n*-NONANE  $\sim 1.2 \cdot 10^3$

Substance	$R_{xN}$ method			<i>n</i> -Heptane = 1.00			<i>n</i> -Octane = 1.00			<i>n</i> -Nonane = 1.00		
	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$
Chloroform	0.056	0.38	0.68	0.338	1.9	0.55	0.137	0.76	0.55	—	—	—
Carbon tetrachloride	0.102	0.67	0.66	0.621	4.9	0.79	0.252	2.3	0.91	—	—	—
Toluene	0.256	2.6	1.0	1.553	13	0.87	0.628	5.3	0.84	—	—	—
Oct-1-ene	0.344	2.1	0.60	2.084	14	0.69	0.847	1.2	0.15	—	—	—
Chlorobenzene	0.464	1.7	0.37	—	—	—	1.141	1.7	0.15	0.464	2.7	0.57
Methyl phenyl ether	0.796	15	1.9	—	—	—	1.957	38	1.9	0.797	13	1.6
Hexane-1-thiol	0.939	9.4	1.0	5.684	63	1.1	2.310	11	0.46	—	—	—

TABLE VIII  
20% SQUALANE/CELITE 100°  $b = 0.324$   $K$  *n*-NONANE  $\sim 0.31 \cdot 10^3$

Substance	$R_{xN}$ method			<i>n</i> -Octane = 1.00			<i>n</i> -Nonane = 1.00			<i>n</i> -Decane = 1.00		
	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$	$R_{xN}$	$P_{xN} \cdot 10^3$	$\sigma_{xN}$
Oct-1-ene	0.414	2.1	0.50	—	—	—	0.415	2.4	0.58	0.196	1.8	0.89
Hexane-1-thiol	1.001	5.1	0.51	2.115	11	0.53	—	—	—	0.475	2.9	0.62
Ethyl phenyl ether	1.503	15	0.98	—	—	—	1.507	16	1.1	0.713	6.8	0.95
2,6-Dimethyl-octa-2,6-diene	1.794	5.5	0.30	—	—	—	1.800	8.4	0.47	0.850	3.4	0.4
Nitrobenzene	2.333	52	2.2	—	—	—	2.342	57	2.4	1.104	22	2.0
Octane-1-thiol	4.497	17	0.39	9.514	38	0.4	—	—	—	2.129	6.6	0.31

TABLE IX  
 20% SQUALANE/CELITE 138°  $b = 0.264$   $K$  *n*-NONANE  $\sim 0.13 \cdot 10^3$

Substance	$R_{20}$ method			$n$ -Decane = 1.00			$n$ -Undecane = 1.00			$n$ -Dodecane = 1.00		
	$R_{20}$	$P_{20} \cdot 10^3$	$\sigma_{20}$	$R_{2N}$	$P_{2N} \cdot 10^3$	$\sigma_{2N}$	$R_{2N}$	$P_{2N} \cdot 10^3$	$\sigma_{2N}$	$R_{2N}$	$P_{2N} \cdot 10^3$	$\sigma_{2N}$
2,6-Dimethyl-octa-2,6-diene	1.629	9.5	0.58	—	—	—	0.479	2.0	0.42	0.261	1.4	0.54
Iodobenzene	2.336	2.3	0.10	—	—	—	0.691	0.82	0.12	0.376	0.58	0.15
Di- <i>n</i> -butyl sulphide	2.743	8.8	0.32	1.494	9.1	0.61	—	—	—	0.441	1.5	0.33
Di- <i>p</i> -ethoxy-benzene	3.671	107	2.9	1.984	56	2.8	—	—	—	0.591	21	3.6
<i>p</i> -Nitrotoluene	4.596	21	0.46	—	—	—	1.358	7.0	0.52	0.738	4.0	0.54
Di- <i>n</i> -butyl disulphide	9.663	23	0.26	—	—	—	2.858	4.9	0.17	1.555	1.8	0.12

TABLE X

MEAN PERCENTAGE STANDARD DEVIATIONS IN RETENTION RATIOS FOR THE MATERIALS STUDIED

Stationary phase	Type of mean %std. deviation*	64.7°	100°	138°
DNP	$\overline{\sigma_{x_9}}$	0.55	0.47	—
	$\overline{\sigma_{xN}}$ opt.	0.52	0.33	—
	$\overline{\sigma_{xN}}$ const.	0.50	0.50	—
Squalane	$\overline{\sigma_{x_9}}$	0.78	0.73	0.59
	$\overline{\sigma_{xN}}$ opt.	0.50	0.83	0.36
	$\overline{\sigma_{xN}}$ const.	0.71	0.87	0.88

\* For the meaning of these symbols see the list of symbols at the end of this paper. It is of interest to record the  $R_{x_9}$  values for the I.U.P.A.C.<sup>11</sup> recommended standards on dinonyl phthalate and squalane at 100°. These are shown in Tables XIV-XV. Apart from the fact that these standards are not related in any way they are clearly inadequate in that they do not cover the retention scale uniformly as shown in the last column of these Tables.

TABLE XI

EFFECT OF THE CHANGE OF STATIONARY PHASE CONCENTRATION AND ALSO THE CHANGE OF SUPPORTING SOLID PHASE ON  $R_{x_9}$  VALUES (64.7°)

Substance	20% DNP/celite	5% DNP/celite	5% DNP/brick dust
Methyl ethyl ketone	0.163	0.175*	0.3*
Butan-1-ol	0.417	0.537*	2.0*
Oct-1-ene	0.426	0.425	0.434
<i>n</i> -Butyl acetate	0.786	0.819*	1.195*
Chlorobenzene	1.267	1.254	1.249
Hexane-1-thiol	1.863	1.847	1.833
Cumene	1.961	1.932	1.910
Anisole	2.594	2.590	2.633

\* Asymmetric peak.

TABLE XII

EFFECT OF THE CHANGE OF STATIONARY PHASE CONCENTRATION AND ALSO THE CHANGE OF SUPPORTING SOLID PHASE ON  $R_{x_9}$  VALUES (100°)

Substance	20% DNP/celite	5% DNP/celite	5% DNP/brick dust
Butan-1-ol	0.438	0.500*	21.7*
Ethylbenzene	1.243	1.216	1.214
Hexan-1-ol	1.952	2.165*	—*
D.H.M.	2.323	2.302	2.299
<i>n</i> -Butyl <i>n</i> -butyrate	2.804	2.837	3.09*
Di-isopropyl disulphide	3.601	3.541	3.496
Phenetole	3.712	3.677	3.716*
Octan-1-ol	8.400	9.14*	—*

\* Asymmetric peak.

TABLE XIII  
ONSET OF COLUMN DEGRADATION WITH TIME OF USAGE AT 100° FOR  
20% SQUALANE/CELITE COLUMN

Substance	$R_{x_0}$ 0-140 h	$R_{x_0}$ 150 h	$R_{x_0}$ 157 h	$R_{x_0}$ 164 h
Oct-1-ene	0.414	0.416	0.417	0.417
Phenetole	1.503	1.519	1.522	1.603
Nitrobenzene	2.333	2.357	2.417	2.673
Octane-1-thiol	4.497	4.501	4.617	4.829

TABLE XIV  
 $R_{x_0}$  VALUES FOR THE I.U.P.A.C. RECOMMENDED STANDARDS  
20% SQUALANE/CELITE, 100°

Substance	$R_{x_0}$	$\log_{10} R_{x_0}$	$\Delta \log_{10} R_{x_0}$
Methyl ethyl ketone	0.071	—1.149	0.322
Benzene	0.149	—0.827	0.157
Isooctane	0.214	—0.670	0.476
Cyclohexanone	0.64	—0.194	0.020
Cyclohexanol	0.67	—0.174	0.047
<i>p</i> -Xylene	0.746	—0.127	0.939
Naphthalene	6.48	0.812	

TABLE XV  
 $R_{x_0}$  VALUES FOR THE I.U.P.A.C. RECOMMENDED STANDARDS  
20% DNP/CELITE, 100°

Substance	$R_{x_0}$	$\log_{10} R_{x_0}$	$\Delta \log_{10} R_{x_0}$
Methyl ethyl ketone	0.216	—0.665	0.011
Isooctane	0.222	—0.654	0.119
Benzene	0.292	—0.535	0.666
<i>p</i> -Xylene	1.353	0.131	0.202
Cyclohexanone	2.155	0.333	0.021
Cyclohexanol	2.261	0.354	0.874
Naphthalene	16.92	1.228	



## SYMBOLS USED

$\delta a$	= correction to calculated column interstitial volume (expressed as distance on chart) required to ensure linear relation between log (retention) and carbon number in the $n$ -paraffin series (applicable from $n$ -butane)
$R$	= retention distance on chart measured from the "air peak"
$\delta \log R$	= deviation of log $R$ from extrapolated linear region of the "log plot"
$R_{N_9}$	= retention of internal standard relative to $n$ -nonane
$R_N$	= retention distance on chart of the internal standard, measured from the "air peak"
$R_9$	= retention distance on chart of $n$ -nonane measured from the "air peak" obtained by extrapolation of "log plot"
$R_{x_9}$	= retention of material being measured relative to $n$ -nonane
$R_{xN}$	= retention of material being measured relative to internal standard
$\epsilon_x$	= % standard deviation in the retention distance on the chart for the material being measured
$\epsilon_N$	= % standard deviation in the retention distance on the chart for the standard
$\epsilon'_{xN}$	= % error in retention ratio due to linear drift in carrier gas flow rate using the internal standard technique
$\alpha$	= change in carrier gas flow rate per unit of time
$F_0$	= carrier gas flow rate at the time of elution of the air peak
$F$	= carrier gas flow rate at any time
$t$	= elution time measured from air peak
$\epsilon'_{xp}$	= % error in the mean retention relative to a primary standard (using two internal standards eluted either side of the material being measured) due to a linear drift in carrier gas flow rate
$\epsilon_{x_9}$	= % standard deviation in $R_{x_9}$ when material being measured, the internal standard, and each paraffin in the calibration are all run on separate chromatograms
$\epsilon_{xN}$	= % standard deviation in $R_{xN}$ when the material being measured and the standard are run on separate chromatograms
$\epsilon_{N_9}$	= % standard deviation in $R_{N_9}$ when each paraffin in the calibration and the standard are all run on separate chromatograms
$\varphi_9$	= probable error in retention distance of $n$ -nonane (obtained by extrapolation or interpolation of the "log plot") divided by the probable error in the retention distance of a $n$ -paraffin which is not eluted too close to the air peak
$i$	= the carbon number of a $n$ -paraffin used in the calibration
$\nu$	= the number of $n$ -paraffins used in the calibration
$m$	= the carbon number of the lowest molecular weight paraffin used in the calibration
$n$	= the carbon number of the highest molecular weight $n$ -paraffin used in the calibration

- $\mathcal{N}$  = the carbon number of the  $n$ -paraffin used as the primary standard (equal to 9 if  $n$ -nonane is taken as primary standard)
- $b$  = the slope of the "log plot" (*i.e.*  $\frac{d \log R_i}{di}$ )
- $R_i$  = retention distance on the chart measured from the air peak for a  $n$ -paraffin with  $i$  carbon atoms
- $n_{\max}$  = the maximum carbon number of a  $n$ -paraffin used in the calibration which is eluted in less than 8 h on a 5 ft.  $\times$  4 mm silicone 704 column with 5 % stationary phase
- $\epsilon_0$  = % standard deviation in retention distance on chart and assumed to be independent of retention distance for materials not eluted close to the air peak
- $I$  = KOVATS' retention index
- $R(x)$  = KOVATS' equivalent of  $R_x$  used in this paper
- $R(P_z)$  = KOVATS' symbol for the retention distance on the chart for a  $n$ -paraffin with  $Z$  carbon atoms
- $R(P_{z+2})$  = KOVATS' symbol for the retention distance on the chart for a  $n$ -paraffin with  $Z + 2$  carbon atoms
- $I'$  =  $I/100$
- $T$  = absolute temperature of column
- $b'$  =  $2.303 b$
- $\gamma^\circ$  = activity coefficient at infinite dilution in the liquid stationary phase taking the pure solute as the standard state
- $\overline{\Delta G}_{x_9}$  = increment in the partial molar free energy of solution at infinite dilution over that for  $n$ -nonane taking the vapour at infinite dilution as the standard state
- $\overline{\Delta H}_{x_9}$  = increment in partial molar enthalpy of solution associated with  $\overline{\Delta G}_{x_9}$  over that for  $n$ -nonane
- $\overline{\Delta S}_{x_9}$  = increment in partial molar entropy of solution associated with  $\overline{\Delta G}_{x_9}$  over that for  $n$ -nonane
- $P_{x_9}$  = observed standard deviation in  $R_{x_9}$
- $P_b$  = observed standard deviation in  $b$
- $\sigma_{x_9}$  = observed % standard deviation in  $R_{x_9}$  using internal standard technique
- $P_{xN}$  = observed standard deviation in  $R_{xN}$  using internal standard technique
- $\sigma_{xN}$  = observed % standard deviation in  $R_{xN}$  using internal standard technique
- $\overline{\sigma}_{x_9}$  = mean percentage standard deviation in  $R_{x_9}$  for all the values quoted in this paper
- $\overline{\sigma}_{xN \text{ opt.}}$  = mean percentage standard deviation in  $R_{xN}$  using a  $n$ -paraffin as internal standard with the closest retention to each material being measured for all the values quoted in this paper
- $\overline{\sigma}_{xN \text{ const.}}$  = mean percentage standard deviation in  $R_{xN}$  using one  $n$ -paraffin as internal standard with a retention in the middle range of retentions measured at each temperature and stationary phase.

## SUMMARY

The reproducibility of retention data in  $R_{x_9}$  units is discussed theoretically. It is shown that high reproducibility should be readily obtainable by using suitable calibration procedures and experimental evidence in support of this is presented for a wide range of compounds.

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