

GAS-LIQUID CHROMATOGRAPHY IN QUALITATIVE ANALYSIS
PART V. THE DETERMINATION OF RELATIVE RETENTIONS
IN R_{x_9} AND RETENTION INDEX UNITS BY MEANS OF SECONDARY
STANDARDS

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INTRODUCTION

Recent proposals¹ have advocated the use of the *n*-alkanes as standards for the determination of relative retention data; the results being expressed in R_{x_9} ² and retention index units³. Though it is possible to use the *n*-alkanes as standards with practically all stationary phases it is often necessary to use other substances as secondary standards, particularly for the analysis of complex mixtures. Methods of calculating R_{x_9} values and retention indices by means of secondary internal standards have therefore been investigated in an attempt to rationalise relative retention data.

EXPERIMENTAL

Chromatograms were obtained using an apparatus consisting of pyrex glass columns (5 ft. long, 4 mm I.D.) with a modified flame ionisation detector⁴. A mixture of hydrogen and nitrogen (3:1 by vol.) was used as carrier gas. This was dried over linde molecular sieve and was allowed to flow continuously in order to ensure reproducible retention data⁵. The column was packed with a 20% w/w mixture of polyethylene glycol-400 (Union Carbide) and acid washed (1% phosphoric acid) 60-70 mesh celite (J.J.'s, Ewell, Surrey). The column was heated by means of a water vapour jacket. Samples for analysis were introduced as dilute solutions in *n*-heptane (Phillips pure grade) by means of stainless steel capillary pipettes. The solutes used were either obtained commercially or synthesised by colleagues at the Natural Rubber Producers' Research Association.

PROCEDURE

In order to calculate the retention of an unknown in R_{x_9} or retention index units by means of a secondary internal standard the retention of the latter must be known in these units. These standards which may be either single substances or members of a homologous series may be calibrated directly or by means of the functional group retention parameters Functional Retention Index (F.R.I.⁶) or ΔMe^7 .

The relative retentions of a number of chemically unrelated substances have been

determined using octan-2-ol, fatty acid methyl esters, and *n*-alkanes as internal standards in order to illustrate and to test the reliability of the proposed calibration procedures.

1. *Determination of the R_{x0} values and retention indices with the *n*-alkanes as standards*

The chromatogram of a convenient calibration series of *n*-alkanes (*n*-decane to *n*-nonadecane) was recorded and the retention distances measured from the point of injection to peak maxima on the recorder chart. The dynamic column dead volume was computed by linearisation of the "log plot"⁸ and the R_{N0} values of the *n*-alkanes calculated by the slope of the "log plot" method⁸.

Chromatograms of solutions containing one solute and two *n*-alkanes, eluted either side of the unknown, were then recorded and the retention distances measured. The mean R_{x0} value was calculated from the corrected retention distances using the expression:

$$R_{x0} = R_{xN} \times R_{N0} \quad (1)$$

where R_{xN} is the retention of the unknown relative to the *n*-alkane standard carbon number *N* and R_{N0} the retention of that *n*-alkane relative to *n*-nonane.

The retention indices of the solutes were calculated from the same corrected retention distances using the expression:

$$I = 100N + 100n \left\{ \frac{\log R_x - \log R_N}{\log R_{N+n} - \log R_N} \right\} \quad (2)$$

where *N* is the carbon number of the first *n*-alkane standard and *N* + *n* that of the second,

R_x the retention distance of the unknown

R_N and R_{N+n} the retention distances of the *n*-alkanes carbon number *N* and *N* + *n*, respectively.

As far as possible consecutive *n*-alkanes were used.

2. *Determination of the R_{x0} values and retention indices with octan-2-ol as standard*

The chromatogram of a mixture of octan-2-ol and two *n*-alkanes, eluted either side of the alcohol, was recorded, the retention distances measured, and the retention index and R_{x0} value determined as described in the preceding section.

Chromatograms of mixtures of octan-2-ol and each of the solutes shown in Table II were then recorded in turn. The retention distances were measured and corrected for the column dead volume. The R_{x0} values and retention indices were calculated using the expressions:

$$R_{x0} = R_{xs} \times R_{s0} \quad (3)$$

where R_{xs} is the retention of the unknown relative to the secondary standard and R_{s0} the retention of the standard relative to *n*-nonane, and

$$I = 100 \left\{ \frac{\log R_{xs}}{b} + I_s' \right\} \quad (4)$$

where I_s' is the effective carbon number of the standard (retention index divided by

100) and b the slope of the n -alkane "log plot" which is assumed to remain constant. This can be obtained from the retentions of two n -alkanes using the expression:

$$b = \frac{\log R_{N+n} - \log R_N}{n} \quad (5)$$

where $N + n$ and N are the carbon numbers of the two n -alkanes.

Equation (3) is derived from eqn. (1) by simple algebra whilst eqn. (4) follows from the relation between retention index and R_{x_0} .

3. Determination of the R_{x_0} values and retention indices with the fatty acid methyl esters as standards

The retentions of the methyl esters were determined using:

- (1) The n -alkanes as internal standards as described in Section 1.
- (2) The functional group retention parameters ΔMe and F.R.I.

The mean ΔMe value for the methyl ester group was calculated from the R_{x_0} values of the five homologues using the expressions:

$$Me = 14.03 \left\{ \frac{\log R_{x_0}}{b} \right\} + 128.25 \quad (6)$$

and

$$\Delta Me = Me - M \quad (7)$$

where M is the molecular weight of the ester.

The mean F.R.I. value was determined from the retention indices by means of the expression:

$$F_{COOMe} = I_{R \cdot COOMe} - I_{R \cdot H} \quad (8)$$

where $I_{R \cdot COOMe}$ and $I_{R \cdot H}$ are the retention indices of the methyl ester and n -alkane corresponding to the alkyl chain of the fatty acid.

In view of the close agreement of the results obtained by the two methods (see Table I) only those calculated using ΔMe and F.R.I. were used subsequently.

Chromatograms of solutions containing one unknown and two methyl esters, eluted on either side of the unknown, were then obtained, the retention distances measured and corrected for the column dead volume. The mean R_{x_0} value of the unknown was calculated by means of eqn. (3) using the retentions of the two esters in turn.

The retention indices of the unknowns were obtained from the same retention distances using the expression:

$$I = 100N + F_{COOMe} + 100n \left\{ \frac{\log R_x - \log R_1}{\log R_2 - \log R_1} \right\} \quad (9)$$

where N is the number of carbon atoms in the alkyl chain of the first methyl ester, n the difference in the carbon numbers of the two ester standards, and R_x , R_1 and R_2 the retention distances of the unknown and the two esters in order of increasing retention.

Though we in this case have used five homologues in order to determine the mean ΔMe and F.R.I. values in normal practice two homologues would be sufficient except for highly polar materials on polar stationary phases, for instance the n -alcohols see Table I.

DISCUSSION AND RESULTS

The greater part of the relative retention data in the literature has been determined using single substances as internal standards. Thus calibration of the standard by the methods described for octan-2-ol under the same column conditions should enable the conversion of the data into more widely acceptable units. The internal standard can be used for the concurrent determination of retention data and absolute concentrations. Though the *n*-alkanes can be used in this respect it is often preferable to use a standard of similar chemical character to the compounds being analysed; particularly when using capillary pipettes in order to minimise errors due to preferential volatilisation during injection. With thermally unstable stationary phases it should be appreciated that any change of the retentive character of the column will cause serious fluctuations of the retentions of the secondary standards relative to the *n*-alkanes. In such cases a different stationary phase should be used.

The errors involved in the direct calibration of a homologous series of secondary standards are minimised if they are chromatographed together with the *n*-alkanes. The calculation of the retentions by means of ΔMe or F.R.I. values requires that these parameters remain constant throughout a homologous series. Whereas the ΔMe values for six series of monosubstituted *n*-alkanes determined using dinonyl phthalate as stationary phase were found to be constant from *n*-propyl onwards⁸, SWOBODA found that the F.R.I. values for the saturated aliphatic aldehydes increased with chain length from *n*-butanal to *n*-decanal.⁹ Thus, it was necessary to check the constancy of these parameters with the polyethylene glycol stationary phase. The functional group retentions of the *n*-alcohols were found to increase from *n*-butanol to *n*-octanol presumably due to electronic interaction between the hydroxyl group and the adjacent methylene groups. The less polar methyl esters, however, remained constant from methyl *n*-butyrate as shown in Table I. Also included in this table are the observed R_{x0} and retention indices and those calculated by means of the functional group retention parameters. Clearly when the effective contribution of the functional group to the retention of a molecule varies throughout a series the observed and calculated retentions deviate seriously particularly with the lower homologues.

The procedure is capable of extension to any column temperature since ΔMe has been found to vary linearly with temperature according to the expression:

$$\Delta Me_{\theta} = \Delta Me_0 + \mu\theta \quad (10)$$

where ΔMe_{θ} and ΔMe_0 are the values at θ° and 0° and μ the temperature coefficient⁹. Thus if ΔMe_0 and μ are known for a homologous series the retentions can be calculated in R_{x0} units at any column temperature for which b is known. F.R.I. should vary linearly for monosubstituted *n*-alkanes for which ΔMe and F.R.I. are simply related¹⁰.

Since this method of predicting the retentions of the secondary standards is dependent upon the constancy of ΔMe and F.R.I. within a homologous series, it is important that the constancy should be established, particularly with strongly polar solutes on polar phases.

The relative retentions of a number of substances have been determined in R_{x0} and retention index units using the *n*-alkanes, methyl esters and octan-2-ol as standards by the methods described in the procedure section. The results (in Table II) reveal

TABLE I

THE FUNCTIONAL GROUP RETENTION PARAMETERS ΔMe AND F.R.I. FOR *n*-ALCOHOLS AND METHYL ESTERS AND COMPARISON OF RETENTIONS CALCULATED USING THESE PARAMETERS WITH OBSERVED DATA

Substance	ΔMe	F.R.I.	Retention in R_{20} units			Retention in retention index units		
			Observed	Calculated*	Percent Error	Observed	Calculated*	δI^{**}
Butan-1-ol	+ 106.28	871.8	7.33	8.20	11.9	1271.8	1293.0	+ 21.2
Pentan-1-ol	+ 107.76	882.4	13.25	14.03	5.9	1382.4	1393.0	+ 10.6
Hexan-1-ol	+ 108.49	887.6	23.27	23.93	2.8	1487.6	1493.0	+ 5.4
Heptan-1-ol	+ 109.06	891.5	40.62	40.83	0.5	1591.5	1593.0	+ 1.5
Octan-1-ol	+ 109.38	894.6	70.55	69.81	1.0	1694.6	1693.0	- 1.6
Methyl <i>n</i> -butyrate	+ 48.67	760.6	2.358	2.377	0.8	1060.6	1062.6	+ 2.0
Methyl <i>n</i> -hexoate	+ 48.90	762.3	6.92	6.93	0.1	1262.3	1262.6	+ 0.3
Methyl <i>n</i> -octanoate	+ 48.87	762.2	20.10	20.14	0.2	1462.2	1462.6	+ 0.4
Methyl <i>n</i> -nonanoate	+ 48.96	762.8	34.39	34.35	0.1	1562.8	1562.6	- 0.2
Methyl <i>n</i> -decanoate	+ 49.01	763.2	58.76	58.59	0.3	1663.2	1662.6	- 0.6
Methyl <i>n</i> -dodecanoate	+ 48.93	762.6	170.30	170.30	Nil	1862.6	1862.6	Nil

* Calculated using mean values of $\Delta Me = + 109.22$ and F.R.I. = 893.0 for the *n*-alcohols and + 48.93 and 762.6 for the methyl esters.

** δI is the difference in observed and calculated retention indices.

TABLE II

RELATIVE RETENTIONS IN R_{20} AND RETENTION INDEX UNITS DETERMINED USING *n*-ALKANES, METHYL ESTERS AND OCTAN-2-ol AS STANDARDS

Substance	Retention in R_{20} units			Retention in retention index units		
	<i>n</i> -Alkanes	Esters	Octan-2-ol	<i>n</i> -Alkanes	Esters	Octan-2-ol
6-Methylhept-5-en-2-one	17.53	17.62	17.48	1436.5	1437.5	1436.0
Phenetole	19.59	19.76	19.56	1457.5	1459.0	1457.1
1-Bromononane	25.75	25.86	25.77	1508.6	1509.5	1508.8
1-Ethyl-2-methylpent-2-enol	30.23	30.28	—*	1538.6	1538.9	—*
Diallyl disulphide	31.64	31.79	—*	1547.0	1548.1	—*
1,1-Dimethylbutyl-2-methyl pent-2-enyl sulphide	36.92	37.10	36.89	1575.9	1577.0	1575.9
Di-isopropyl trisulphide	38.30	38.35	38.18	1583.0	1583.3	1582.7
<i>n</i> -Decylaldehyde	40.20	40.40	40.16	1592.1	1593.0	1591.7
N,N-Methyl-ethyl-aniline	58.48	58.88	58.55	1662.5	1663.5	1662.5
Di-(2-methylpent-2-enyl)sulphide	66.94	67.48	66.85	1687.7	1688.7	1687.4
N,N-Diethylaniline	67.61	67.59	67.30	1689.5	1689.4	1688.6
Percentage standard deviation	—	0.53	0.23	—	—	—
Standard deviation in retention index units	—	—	—	—	0.9	0.4

* Not resolved from octan-2-ol peak.

It is normal practice to express as a percentage standard deviation for R_{20} and as a standard deviation in retention index units for retention index.

an excellent agreement between the values obtained using the various standards particularly as only one *n*-alkane calibration was recorded; the value of *b* being assumed to remain constant during the period of the investigation (approximately 5 days). Recent work¹¹ using vapour jacket heating has shown that fluctuations of barometric pressure have a small but significant effect on *b* and R_{x_0} and to a lesser extent retention index. Therefore one would expect an even better agreement between the retentions obtained using the different standards with good electrical heating where a control of better than $\pm 0.1^\circ$ is possible.

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SUMMARY

Procedures are described whereby relative retentions can be determined in R_{x_0} and retention index units by means of internal standards other than *n*-alkanes. Such procedures are important as they enable the calculation of all relative retention data in either of two simply related units.

REFERENCES

- ¹ M. B. EVANS, G. M. C. HIGGINS AND J. F. SMITH, to be published.
- ² J. F. SMITH, *Chem. Ind. (London)*, (1960) 1024.
- ³ E. KOVATS, *Helv. Chim. Acta*, 41 (1958) 1915.
- ⁴ J. F. SMITH, in R. P. W. SCOTT (Editor), *Gas Chromatography 1960*, Butterworths, London, 1960, p. 114.
- ⁵ M. B. EVANS AND J. F. SMITH, to be published as Part VI.
- ⁶ P. A. T. SWOBODA, in M. VAN SWAAY (Editor), *Gas Chromatography 1962*, Butterworths, London, 1962, p. XXX.
- ⁷ M. B. EVANS AND J. F. SMITH, *Nature*, 190 (1961) 905.
- ⁸ M. B. EVANS AND J. F. SMITH, *J. Chromatog.*, 9 (1962) 147.
- ⁹ M. B. EVANS AND J. F. SMITH, *J. Chromatog.*, 8 (1962) 303.
- ¹⁰ P. A. T. SWOBODA, in M. VAN SWAAY (Editor), *Gas Chromatography 1962*, Butterworths, London, 1962, p. 282.
- ¹¹ M. B. EVANS, to be published.