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RETENTION INDICES AS A TOOL FOR THE PREDICTION OF CHROMATOGRAPHIC CONDITIONS FOR ACHIEVING THE SEPARATION OF MIXTURES OF ORGANIC COMPOUNDS

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SUMMARY

The earlier derived equation relating the minimum difference in retention indices (ΔI_{\min}) with the column efficiency (N') necessary to achieve the desired level of separation of sorbates has been used to establish the chromatographic conditions for the analysis of some multi-component insect sex pheromones. The b_z parameters [slope of the equation linearly relating the logarithm of the adjusted retention time of a standard to the carbon chain length (z)] have been calculated for homologous series of n -alkanes, alkan-1-ols and the corresponding acetates on columns with stationary phases of different polarity. Nomograms are given for the rapid evaluation of the chromatographic conditions required for the separation of two (or more) sorbates with known retention indices.

INTRODUCTION

The development of optimal chromatographic procedures for the separation and quantification of mixtures of organic compounds generally involves a search for a suitable stationary phase and calculation of the column efficiency necessary to achieve a satisfactory resolution ($R \geq 1.0$) of the sorbates. Usually this procedure is carried out by trial and error and is time consuming. Hence the development of theoretical models for the prediction of chromatographic conditions for the analysis of different mixtures is essential.

Useful qualitative information can be obtained from the Rohrschneider¹ or McReynolds constants². However, these parameters do not indicate the actual resolving potential of the stationary phase. The use of the Purnell equation^{3,4} relating the column efficiency (effective plate number, N') to sorbent selectivity (α) and resolution (R):

$$N' = 16R^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \quad (1)$$

is limited because of the absence of data on relative retention times (α) on different stationary phases for most compounds.

It is more appropriate to develop such models on the basis of logarithmic retention indices, which are known for many compounds and are regularly published in the Chromatographic Data Section of the *Journal of Chromatography* and in handbooks^{5,6}. Earlier^{7,8}, several relationships between column efficiency (N'), resolution (R) and difference in retention indices of sorbates (ΔI) have been proposed. However, the accuracy of calculation of these equations is not high enough.

Recently⁹ we derived a modified equation that approximates with good accuracy the Purnell equation (eqn. 1) (the error being less than 2% relative for both capillary and packed columns):

$$N' = 16R^2 \left[\frac{10^4 \log^2 e}{b_z^2 (\Delta I)^2} + \frac{10^2 \log e}{b_z (\Delta I)} \right] \quad (2)$$

where $\Delta I = I_2 - I_1$ is the difference in retention indices of two sorbates, N' is the effective plate number, e is the basis of natural logarithms, $b_z = \log t'_{(z+1)}/t'_z$ and t'_z and $t'_{(z+1)}$ are the adjusted retention times of two members of standard homologous series with carbon chain lengths z and $z+1$, respectively.

We report here practical applications of eqn. 2 for the selection of suitable stationary phases and for the prediction of the resolution of mixtures of organic compounds with known retention indices. In addition, nomograms relating the minimum retention index differences (ΔI_{\min}) to column efficiency (N') for various b_z values are given.

EXPERIMENTAL

A Tsvet Model 5 gas chromatograph equipped with a flame ionization detector and standard 2 m \times 2 mm I.D. glass columns was used to determine retention data. The stationary phases used were the silicones OV-1, OV-17, OV-25 and OV-225, diethylene glycol succinate (DEGS) and 1,2,3-tris-(β -cyanoethoxy)propane (TRIS), all 10% (w/w) on 80–100-mesh Chromosorb W AW DMCS (Applied Science Labs.). Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min.

A Chrom Model 5 gas chromatograph (Czechoslovakia) equipped with a flame ionization detector, a splitter with a splitting ratio of 1:50 and a stainless-steel capillary column (50 m \times 0.25 mm I.D.) coated with TRIS and a glass capillary column (30 m \times 0.2 mm I.D.) coated with DEGS was also used. Nitrogen was used as the carrier gas at a flow-rate of 4 ml/min. The column temperature was 130°C.

The gas hold-up time was determined according to the method of Peterson and Hirsch¹⁰ by the use of homologous series of acetates of alkan-1-ols.

RESULTS AND DISCUSSION

For the practical application of eqn. 2 it is important to know b_z values which are dependent on temperature, on the type of standards used for the calculation of retention indices and on the type of stationary phase. Table I gives b_z values and their temperature coefficients determined for several stationary phases of different polarity at 130°C. These results indicate a marked decrease in b_z with increasing stationary phase polarity. Thus, with equal ΔI values for two sorbates on columns with polar and non-polar stationary phases, a better separation can be achieved on the latter column.

TABLE I

b_z VALUES FOR VARIOUS STATIONARY PHASES CALCULATED BY THE USE OF THREE TYPES OF STANDARDS

Experimental conditions: temperature, 130°C; glass columns (2 m × 2 mm I.D.) packed with Chromosorb W AW DMCS (80–100 mesh) containing 10% (w/w) of stationary phase; stainless-steel capillary column (50 m × 0.25 mm I.D.) coated with TRIS; glass capillary column (30 m × 0.20 mm I.D.) coated with DEGS.

Stationary phase	Polarity of stationary phase, P^*	Type of standard used for the calculation					
		<i>n</i> -Alkanes		Alkan-1-ols		Acetates of alkan-1-ols	
		b_z	$-10 \cdot \frac{\Delta b_z}{\Delta T}$	b_z	$-10 \cdot \frac{\Delta b_z}{\Delta T}$	b_z	$-10 \cdot \frac{\Delta b_z}{\Delta T}$
OV-1	222	0.235	0.016	0.216	0.012	0.230	0.012
OV-17	884	0.225	0.020	0.225	0.014	0.223	0.012
OV-25	1175	0.220	0.017	0.220	0.012	0.222	0.012
OV-225	1813	0.195	0.020	0.188	0.014	0.190	0.013
DEGS	3543	0.186	0.020	0.168	0.011	0.168	0.012
TRIS	4145	0.189	0.020	0.163	0.010	0.163	0.011

* P = Sum of the first five McReynolds constants taken from ref. 2.

It should be noted that in order to obtain accurate results for the prediction of separation it is expedient to determine b_z values at the desired temperature for each column which is to be used for the analysis. It is conditioned by the fact that the column-to-column reproducibility of b_z values for the same stationary phase is not high enough. For example, an increase in the ratio of polar stationary phase (DEGS) to support (Chromosorb W AW DMCS) from 3 to 12% (w/w) at 130°C results in a decrease in b_z from 0.187 to 0.179 and from 0.241 to 0.206 for homologous series of acetates of alkan-1-ols and *n*-alkanes, respectively.

At the same time, the approximate estimation of chromatographic parameters can be carried out successfully using for non-polar stationary phases ($P = 0-500$) the b_z value determined for OV-1, for medium polarity stationary phases ($P = 500-1400$) b_z for OV-17, for polar stationary phases ($P = 1400-2100$) b_z for OV-225 and for highly polar phases ($P \geq 2100$) b_z for DEGS. The procedure can be further facilitated by the use of nomograms constructed on the basis of eqn. 2 (Figs. 1 and 2). These nomograms relate the minimum difference in retention indices (ΔI) to the

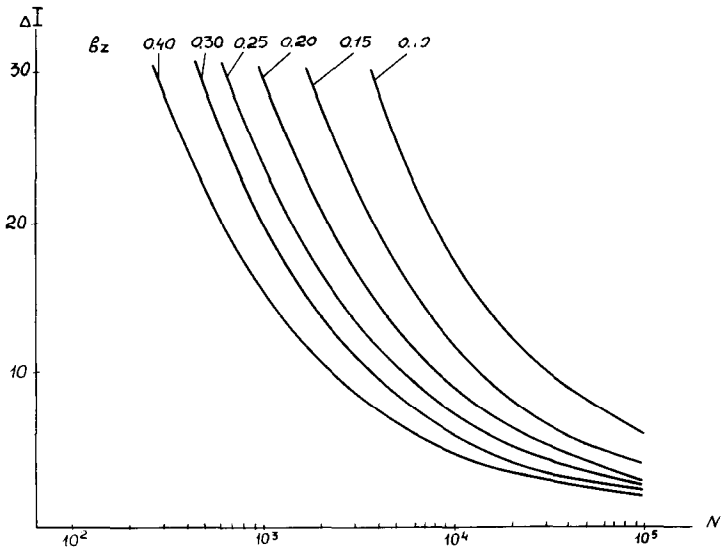


Fig. 1. Nomograms relating the minimum difference in retention indices ($\Delta I = 0-30$) of sorbates to column efficiency (N') necessary to achieve satisfactory resolution ($R \geq 1.0$) for various b_z values.

effective plate number (N') required to obtain the desired level of separation ($R \geq 1.0$) for various b_z values. If there is no curve corresponding to the obtained b_z values in the nomograms, the required parameters can be evaluated as an average of two neighbouring values.

Let us now consider some examples of the practical application of eqn. 2.

Example 1. Calculation of the minimum difference in retention indices (ΔI_{\min}) necessary to achieve the desired level of separation ($R \geq 1.0$) of sorbates on packed and capillary columns.

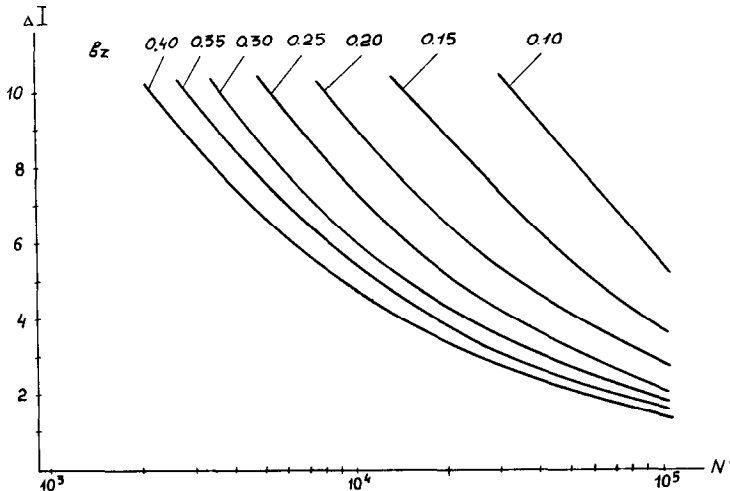


Fig. 2. Nomograms relating the minimum difference in retention indices ($\Delta I = 0-10$) of sorbates to column efficiency (N') necessary to achieve satisfactory resolution ($R \geq 1.0$) for various b_z values.

As a rule, the chromatographic analysis is carried out at temperatures higher than 60°C and, therefore, the maximum value of b_z can be assumed to be that of the non-polar stationary phase OV-1 ($b_z = 0.35$) as the increase in polarity of the stationary phase results in a decrease in b_z , as was shown in Table I. Assuming that the maximum efficiencies of packed and capillary columns are less than 6000 and 100 000 effective plates, respectively, we obtain the following: to obtain the desired separation ($R \geq 1.0$) ΔI should be greater than 6.5 retention index units (I.U.) for packed columns and not less than 1.6 I.U. for capillary columns. An increase in temperature from 60 to 130°C results in a decrease in b_z from 0.35 to 0.23 for OV-1 using acetates of alkan-1-ols as standards. In this instance, with the help of nomograms (Fig. 2), we find that $\Delta I_{\min} \geq 10$ for packed columns and $\Delta I_{\min} \geq 2.8$ for capillary columns.

Example 2. Selection of chromatographic conditions for the analysis of the sex pheromone of *Spodoptera littoralis*, which consists of a mixture of acetates of (Z,E)-9,12-tetradecadien-1-ol and (Z,E)-9,11-tetradecadien-1-ol (5:95)¹¹.

Insertion of ΔI values of these sorbates and b_z values from Table I in eqn. 2 gives the efficiency required to achieve the desired level of separation ($R \geq 1.0$) on columns with various stationary phases (Table II). As can be seen from these data, it is expedient to use packed columns with a polar stationary phase, OV-225 or DEGS, for the analysis of these compounds. In order to evaluate the resolution of these sorbates, we insert the values of the efficiency of available packed columns (N'_{act}) and ΔI values into eqn. 2. As can be clearly seen from Table II, the theoretically predicted and experimentally determined resolution factors (R) are in good agreement.

Example 3. Selection of conditions for the chromatographic analysis of the sex pheromone of *Cryptophlebia leucotreta*, which is a mixture of acetates of (E)-8-decen-1-ol, (Z)-dodecen-1-ol and dodecan-1-ol (10:10:1)¹².

Inserting b_z values from Table I and differences in acetate retention indices (ΔI) (i.e., calculated using acetates of alkan-1-ols as standards) in eqn. 2, we calculate the required column efficiency for different stationary phases (Table III). It follows from the results obtained that for the analysis of these compounds capillary columns

TABLE II

COMPARISON OF PREDICTED AND EXPERIMENTALLY DETERMINED PARAMETERS FOR GLC ANALYSIS OF THE SEX PHEROMONE OF *SPODOPTERA LITTORALIS*

Experimental conditions as in Table I.

Chromatographic parameters	Stationary phase			
	OV-1	OV-17	OV-225	DEGS
Retention indices of acetates of:				
(Z,E)-9,12-Tetradecadien-1-ol (1)	1396	1465	1510	1576
(Z,E)-9,11-Tetradecadien-1-ol (2)	1426	1498	1558	1654
Difference in retention indices, ΔI (1,2)	30	33	48	78
Required column efficiency, N'_r	734	652	439	229
Actual column efficiency, N'_{act}	800	680	700	600
Predicted resolution, R_c (1,2)	1.04	1.02	1.26	1.62
Experimental resolution, R_c (1,2)	1.00	1.00	1.12	1.50

TABLE III

COMPARISON OF PREDICTED AND EXPERIMENTALLY DETERMINED PARAMETERS FOR GLC ANALYSIS OF THE SEX PHEROMONE OF *CRYPTOPHLEBIA LEUCOTRETA*

Experimental conditions as in Table I.

Chromatographic parameters	Stationary phase			
	OV-1	OV-25	DEGS	TRIS
Retention indices of acetates of:				
Dodecan-1-ol (3)	1200	1200	1200	1200
(<i>E</i>)-8-Dodecen-1-ol (4)	1190.5	1218.3	1263	1257
(<i>Z</i>)-8-Dodecen-1-ol (5)	1195.6	1220.9	1276.5	1289
Difference in retention indices:				
ΔI (3,4)	9.5	18.3	63	57
ΔI (4,5)	5.1	2.6	13.5	32
Required column efficiency, N'_r	22 525	91 786	6173	1242
Actual column efficiency, N'_a	—	—	7200	2300
Predicted resolution:				
R_c (3,4)	—	—	4.63	2.33
R_c (4,5)	—	—	1.08	1.85
Experimental resolution:				
R_e (3,4)	—	—	4.50	2.22
R_e (4,5)	—	—	1.01	1.71

coated with highly polar stationary phases should be used. Inserting the values of the efficiency (N'_{act}) of available capillary columns and b_z and ΔI values for the stationary phases TRIS and DEGS in eqn. 2, we calculate the resolution factors of these sorbates. As can be seen from Table III, the predicted and experimentally determined resolution factors (R) show good agreement.

CONCLUSIONS

The equation relating the minimum difference in retention indices (ΔI_{min}) to column efficiency (N') has been successfully applied for the selection of conditions for the gas-liquid chromatographic analysis of multi-component insect sex pheromones. The predicted and experimentally determined resolution factors (R) have been shown to correlate well.

Nomograms useful for the rapid evaluation of ΔI_{min} and the column efficiency (N') necessary to achieve the desired level of separation have been constructed on the basis of eqn. 2. The minimum differences in retention indices (ΔI_{min}) of sorbates required to obtain separations with $R \geq 1.0$ on packed and capillary columns at 60 and 130°C have been calculated with the help of these nomograms.

It is desirable to present the retention indices of sorbates together with b_z parameters of stationary phase and of homologous series of standards used for the calculation. Such a set of parameters would facilitate the prediction of the separation of sorbates on various columns.

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