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APPLICATION OF THE UNIVERSAL EQUATION FOR LIQUID CHROMATOGRAPHY TO THE CALCULATION OF THE RETENTION PARAMETERS FOR 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES OF CARBONYL COMPOUNDS

N. I. SVETLOVA*, L. I. SOKOLOVA, D. N. GRIGORYEVA and R. V. GOLOVNYA

A.N. Nesmeyanov Institute of Organo-Element Compounds, USSR Academy of Sciences, Ul. Vavilova 28, Moscow (U.S.S.R.)

SUMMARY

The retention parameters of 2,4-dinitrophenylhydrazone derivatives of *n*-aldehydes, alk-2-enals and alkan-2-ones were determined under conditions of reversed-phase high-performance liquid chromatography (RP-HPLC). The coefficients of the universal equation first suggested for gas-liquid chromatography and of linear equations for the relationship between the logarithms of the adjusted retention time or capacity factor and the homologue number were computed for each of the homologous series. It was found that the accuracy of the retention parameters calculated by the universal equation is one to two orders of magnitude higher than that obtained with the linear equations. The coefficients of the universal equation can be used in a computer program for identification of carbonyl compounds in complex mixtures under the conditions of RP-HPLC.

INTRODUCTION

Carbonyl compounds occur widely in samples of biological and environmental interest. The lability of such carbonyl compounds complicates their analysis so they are usually transformed into their derivatives and analysed by in high-performance liquid chromatography (HPLC)^{1,2}. Methods for identification of organic compounds in multicomponent mixtures are now being developed. Among these are ways of prediction of the retention parameters depending on the structure of the compounds under investigation, the alkyl chain length of the bonded phase and the eluent composition³⁻⁷.

Nakamura *et al.*⁵ analysed the homologous series of C₁-C₁₀ *n*-aldehydes in the form of their 2,4-dinitrophenylhydrazone (2,4-NDPH) derivatives by HPLC and showed graphically that there existed a linear relationship between the logarithm of the adjusted retention time and the carbon number of the molecules, though they did not specify the accuracy of this relationship. A linear dependence of the logarithm of the capacity factor on the carbon number of the DNPH derivatives of C₅-C₁₀ *n*-alkanals, alk-2-enals and alk-2,4-dienals was also found graphically; however, the

data for the first members of these series have not been reported⁶. Linear equations were suggested⁷ to describe a relationship between the logarithm of the capacity factor and the carbon number for homologues from the two series of *n*-aldehyde and alkan-2-one DNPH derivatives on a Nucleosil C₁₈ column. However, capacity factors calculated on the basis of the equations are characterized by considerable standard deviations for C₁–C₁₂ *n*-aldehyde DNPH derivatives when using methanol–water (4:1) as an eluent.

Recently⁸, a universal equation for calculation of retention data in gas–liquid chromatography (GLC) has been suggested and supported thermodynamically. This equation allows one to calculate the retention parameters (retention indices, logarithms of adjusted retention times and retention volumes) for all the members of an homologous series including the first ones

$$Z = \alpha + \beta m + (\gamma \cdot \log m)/m + \xi/[m(m-2)^2 + 0.1] \quad (1)$$

where $Z = I$ (Kováts retention index), or $\log t'$ (adjusted retention time) or $\log V_g$ (specific retention volume), m is the number of the homologue in the series, α , β , γ and ξ are coefficients. The applicability of eqn. 1 to the identification of organic compounds has been demonstrated in GLC with a large body of experimental data; it is employed in computer programs^{9,10}.

In the present work, the possibility of application of eqn. 1 under conditions of reversed-phase HPLC has been investigated for three homologous series of 2,4-DNPH derivatives, *n*-aldehydes, alk-2-enals and alkan-2-ones.

EXPERIMENTAL

Materials

Aliphatic aldehydes, alk-2-ene aldehydes and alkan-2-ones were synthesized according to the described procedures^{11–13} and converted into their 2,4-dinitrophenylhydrazones in the usual manner¹⁴. The latter were twice recrystallized from ethanol. The melting points of the hydrazones coincided with the literature data.

Methanol was purified from possible carbonyl impurities by refluxing with 2,4-dinitrophenylhydrazine in the presence of freshly distilled trichloroacetic and then twice distilled.

Instruments

The determination of carbonyl 2,4-DNPH standards was carried out on a Yanako L 2000L liquid chromatograph equipped with an UV detector operating at 254 nm. The operating conditions were: column, 50 × 0.4 cm; packing, Yanopack ODS; mobile phase, methanol–water (80:20); flow-rate, 1 ml/min; pressure 170–180 kg/cm².

The eluent was degassed prior to analysis. A 5- μ l sample introduced to the chromatograph comprised a methanolic solution containing 40–80 ng of each of the substances under investigation. A 25- μ l Hamilton syringe was used. In calculation of the adjusted retention times, three replicate measurements were made for each of the substances.

Data processing

The adjusted retention times of the substances were calculated as described^{1,5}. The coefficients of equations of the type 1 were calculated by the least squares method with the use of a Texas Instruments TI-59 computer (Houston, TX, U.S.A.), and are listed in Table I together with their standard deviations. Experimental values and those calculated by eqn. 1 are presented in Table II.

DISCUSSIONS

The description of the retention parameters of organic compounds in an homologous series by means of correlation equations is especially useful for the determination of their identity. Earlier, graphical relationships and linear equations were used in reversed-phase HPLC to describe the dependence of the retention parameters on the number of carbon atoms in the homologues. The retention parameters employed were the logarithms of the adjusted retention time or the capacity factors⁵⁻⁷. However, the accuracy of the equations reported previously is not high enough for the calculated parameters to be used for reliable identification of every homologue in a series, which is probably due to inappropriate selection of the optimum form of the equations.

We treated our values of the adjusted retention times obtained for the three homologous series of *n*-alkanal, alk-2-enal and alkan-2-one DNPH derivatives by assuming that there is a relationship of the type 1 under the conditions of reversed-phase HPLC. For each series, we calculated the coefficients for the universal equation and, for comparison, linear equations for the logarithms of the adjusted retention time, $\log t'$ and the logarithms of the capacity factor, $\log k'$, as a function of the homologue number, but not on the carbon number of the molecule since *m* is a parameter common to all of the homologous series. The coefficients of the universal eqn. 1 and the standard deviations, *s*, of $\log t'$ and $\log k'$ for both universal and linear equations are presented in Table I. Comparison of the standard deviations of the calculated values from the experimental $\log t'$ and $\log k'$ values shows that the *s* values for the universal equation are an order of magnitude lower than those for

TABLE I

COEFFICIENTS AND STANDARD DEVIATIONS OF $\log t'$ AND $\log k'$ CALCULATED BY THE UNIVERSAL OR LINEAR EQUATIONS FOR CARBONYL COMPOUND 2,4-DNPHs UNDER CONDITIONS OF REVERSED-PHASE HPLC

Homologous series	Calculated parameter	Coefficients of the universal equation				Standard deviations	
		α	β	$-\gamma$	ξ	S_{univ}	S_{lin}
<i>n</i> -Aldehydes	$\log t'$	1.179484	0.160522	0.438574	0.004761	0.007	0.021
Alk-2-enals		1.387802	0.165630	0.067676	0.003173	0.003	0.010
Alkan-2-ones		1.316531	0.157890	0.517012	-0.008460	0.006	0.050
<i>n</i> -Aldehydes	$\log k'$	0.177149	0.169855	0.422435	0.004661	0.004	0.015
Alk-2-enals		0.410112	0.165650	0.068417	0.003180	0.003	0.010
Alkan-2-ones		0.316534	0.157889	0.517020	-0.008457	0.006	0.050

TABLE II

EXPERIMENTAL AND CALCULATED $\log t'$ and $\log k'$ VALUES OBTAINED BY THE UNIVERSAL EQUATION FOR CARBONYL COMPOUND 2,4-DNPHs UNDER CONDITIONS OF REVERSED-PHASE HPLC

Homologous series	Homologue number, <i>m</i>	$\log t'_{\text{expt.}}$	$\log t'_{\text{calc.}}$	$\log k'_{\text{expt.}}$	$\log k'_{\text{calc.}}$
Acetaldehyde	1	1.3444	1.3443	0.3424	0.3512
<i>n</i> -Propanal	2	1.4814	1.4813	0.4814	0.4999
<i>n</i> -Butanal	3	1.6053	1.5956	0.6074	0.6238
<i>n</i> -Pentanal	4	1.7528	1.7567	0.7520	0.7941
<i>n</i> -Hexanal	5	1.9101	1.9213	0.9164	0.9679
<i>n</i> -Heptanal	6	2.0846	2.0860	1.0846	1.1418
Prop-2-enal	1	1.5563	1.5563	0.5786	0.5786
But-2-enal	2	1.7404	1.7406	0.7627	0.7629
Pent-2-enal	3	1.8808	1.8768	0.9031	0.8991
Hex-2-enal	4	2.0354	2.0409	1.0577	1.0632
Hept-2-enal	5	2.2068	2.2068	1.2292	1.2291
Oct-2-enal	6	2.3747	2.3730	1.3971	1.3953
Propanone-2	1	1.4669	1.4667	0.4668	0.4667
Butanone-2	2	1.4698	1.4699	0.4698	0.4699
Hexanone-2	4	1.8751	1.8682	0.8751	0.8682
Heptanone-2	5	2.0294	2.0328	1.0294	1.0328
Octanone-2	6	2.1875	2.1963	1.1875	1.1962
Decanone-2	8	2.2563	2.5210	1.5263	1.5210

the linear equation. Such an accuracy of computation of $\log t'$ and $\log k'$ for 2,4-DNPH derivatives of carbonyl compounds has been attained for the first time and follows from the analysis of our results and those reported earlier⁵⁻⁷. The experimental values of $\log t'$ and $\log k'$ and those obtained with eqn. 1 for the substances investigated are given in Table II. The high accuracy of the universal equation justified its use for the description of the relationship between the retention parameters of carbonyl 2,4-DNPHs and the homologue number, *m*, under the conditions of reversed-phase HPLC.

It was interesting to determine whether eqn. 1 would be helpful in processing the experimental data of Vigh *et al.*⁷ and then to compare the calculation errors in $\log k'$ obtained from eqn. 1 and from the linear equation suggested⁷. For this purpose we calculated the coefficients for eqn. 1 for the capacity factor of *n*-aldehydes 2,4-DNPH derivatives separated on a column packed with Nucleosil C₁₈ (eluent, 74,8% aqueous methanol). We obtained the equation:

$$\log k' = -0.067707 + 0.164656 m - 0.348891 \frac{\log m}{m} + \frac{0.006418}{(m-2)^2 + 0.1} \quad (2)$$

We then calculated the capacity factors for the *n*-aldehyde derivatives and their standard deviations by use of eqn. 2. The *s* value was found to be 0.008 which is two

orders of magnitude less than that obtained with the linear equation⁷. This also confirmed the higher accuracy of eqn. 1 compared with the linear one.

Thus, the universal eqn. 1 first applied in GLC may be recommended for calculation of the retention parameters of 2,4-DNPH derivatives of various carbonyl compounds and for establishing the identity of the homologous under conditions of reversed-phase HPLC.

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