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REVERSED-PHASE LIQUID CHROMATOGRAPHIC ELUTION CHARACTERISTICS OF SUBSTITUTED N-ETHYLBENZAMIDES

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SUMMARY

The reversed-phase liquid chromatographic retention of sixteen N-ethylbenzamides substituted with methyl, methoxy or phenyl groups at the 4-phenyl position and/or at the 2-ethyl position was investigated using two different octadecyl phase columns and a phenyl phase column with water-methanol solvent mixtures. For isomeric amides an increased retention was obtained for the isomer having the larger substituent at the 4-phenyl position. Satisfactory linear correlations were obtained by plotting $\log k'$ obtained on one column against $\log k'$ on a second column at the same or different eluent compositions. This suggests that quantitative structure-retention relationships can be transformed from one reversed-phase system to another. The molecular connectivity indices, χ , to third order were calculated for the amides, and a high degree of correlation was observed between them and the measured $\log k'$.

INTRODUCTION

The effect of chemical structure on retention in liquid chromatography is a topic of much current research¹⁻⁹. Wells and co-workers¹⁻³ have recently published a complete study on the retention of C₁-C₅ N-alkylbenzamides using different octadecyl bonded phase columns. The N-alkylbenzamides were formed by the reaction between benzoyl chloride and all possible primary aliphatic amines containing 1-5 carbon atoms. Wells and co-workers³⁻⁵, like many others⁶⁻⁹, have used molecular connectivity indices to correlate chromatographic parameters with molecular structure.

The molecular connectivity indices reflect the shape and atomic interactions of a molecule. Detailed discussions of this concept and of its calculation have been given by Kier and Hall¹⁰ and Wells *et al.*³. When the nature of the atom is not taken into consideration the index is referred to as the connectivity level, χ , while if such characteristics are allowed for the index is referred to as the valence level, χ^v . Connectivity indices have been extended to include indices of different orders (the order being the number of bonds involved in the subgraph of the molecule and denoted by a left-side superscript), as well as subgraphs composed of paths, clusters, path/clusters and chains which are denoted by the subscripts p, c, pc and ch, respectively. Wells and co-workers³⁻⁵ have used such extended indices.

This paper reports the reversed-phase chromatographic properties of sixteen *N*-ethylbenzamides on three different stationary phases. Molecular connectivity indices to the third order were calculated and correlated with log capacity factor (k'). The effect of the stationary phase on retention is also discussed.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 1084B high-performance liquid chromatograph equipped with a 79875A variable wavelength spectrophotometer detector was used. Absorbance spectra were determined in the UV-region, 200–320 nm, on a Kontron Uvikon 820 spectrophotometer.

Reagents and chemicals

Ethylamine hydrochloride, propylamine hydrochloride, β -phenylethylamine, 4-toluoyl chloride and biphenyl-4-carbonyl chloride were obtained from Fluka (Buchs, Switzerland). 4-Methoxybenzoyl chloride, methylene chloride, benzene, acetone, sodium nitrate and sodium sulphate were obtained from E. Merck (Darmstadt, G.F.R.). 2-Methoxyethylamine was from EGA-Chemie (Steinheim, G.F.R.). Benzoyl chloride was from BDH (Poole, Great Britain). Light petroleum (b.p. 40–60°C) was from May & Baker (Dagenham, Great Britain) and was distilled before use. Freshly distilled diethyl ether was from Kemira Corporation (Vihtavuori, Finland). High-performance liquid chromatographic (HPLC) grade methanol was from Orion Corporation (Espoo, Finland) and water was distilled and deionized. Amines and benzoyl chlorides were pro analysis grade (except toluoyl chloride which was practical), and were used without further purification.

Preparation of derivatives

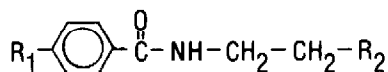
The appropriate amine or amine hydrochloride (3 mmol) was mixed with 100 ml of 2 *N* sodium hydroxide in a three-necked flask equipped with a magnetic stirrer, reflux condenser and a funnel. Benzoyl chloride or the appropriate 4-substituted benzoyl chloride (1.5 molar excess) was added dropwise from the funnel. The resulting mixture was stirred for 1 h at room temperature, then extracted twice with diethyl ether or methylene chloride. The combined organic layers were washed twice with water, dried with sodium sulphate and evaporated. The residue was purified by recrystallization from light petroleum–benzene (1:2, v/v).

Columns

A phenyl bonded phase and two different octadecyl bonded phase analytical HPLC columns were used: a 17 cm \times 4.0 mm I.D. stainless-steel column packed with Spherisorb S5 ODS2 (5 μ m; Phase Separations, Queensferry, Great Britain), a 20 cm \times 4.0 mm I.D. stainless-steel column packed with μ Bondapak C₁₈ (10 μ m; Waters Assoc., Milford, MA, U.S.A.) and a 25 cm \times 4.0 mm I.D. stainless-steel column packed with Nucleosil 7C₆H₅ (7 μ m; from Macherey, Nagel & Co., Düren, G.F.R.). The columns were packed by a slurry technique using acetone as the suspending medium for the octadecyl phase and water–ethanol (58:42) for the phenyl phase. Five grams of packing material were mixed with 50 ml of the appropriate solvent in a

TABLE I

ABSORPTION MAXIMA AND MOLAR ABSORPTION COEFFICIENTS FOR DERIVATIVES INVESTIGATED



Compound No.	R ₁	R ₂	λ _{max} (nm)	ε _{λ_{max}} (cm ⁻¹ M ⁻¹)
1	H	H	224	10,300
2	H	CH ₃	224	10,300
3	H	OCH ₃	225	10,200
4	H	C ₆ H ₅	225	13,900
5	CH ₃	H	234	13,700
6	CH ₃	CH ₃	235	12,700
7	CH ₃	OCH ₃	235	13,700
8	CH ₃	C ₆ H ₅	235	14,400
9	OCH ₃	H	251	15,600
10	OCH ₃	CH ₃	251	15,300
11	OCH ₃	OCH ₃	251	15,900
12	OCH ₃	C ₆ H ₅	251	21,100
13	C ₆ H ₅	H	266	24,800
14	C ₆ H ₅	CH ₃	266	25,100
15	C ₆ H ₅	OCH ₃	267	24,000
16	C ₆ H ₅	C ₆ H ₅	267	26,100

ultrasonic bath and the packing was performed with a Shandon HPLC packing pump using 200 ml methanol at 350 bar pressure. Both octadecyl phases were end-capped so that no free silanol groups were left.

Chromatographic procedures

The eluent was pumped isocratically at a flow-rate of 1.0 ml/min and the oven temperature was 40°C. The column dead volume was taken as the elution volume for a 2-μl injection of an aqueous solution containing sodium nitrate, with the UV detector operating at 254 nm and 0.01 a.u.f.s. Sufficiently dilute samples were prepared to give the smallest detectable peaks (<20% of scale). The UV detector was operated at the wavelength of maximum absorbance for each of the compounds (Table I). Capacity factors were calculated in the usual manner and at least two injections were made in each case.

The specific gravity was measured for each batch of HPLC solvent mixture. The methanol content of solvent mixtures was checked by a pycnometric method at 20 ± 0.02°C. Duplicate analysis were performed. The concentrations of water and methanol in the eluent are given as volume percentages.

RESULTS AND DISCUSSION

Table I lists the N-ethylbenzamide derivatives investigated together with their absorption maxima and molar absorption coefficients. All compounds are white solids except N-2-methoxyethyl-4-methoxybenzamide (11) which is oily.

TABLE II

PARAMETERS OF LOG k' VS. METHANOL CONTENT FOR SPHERISORB S5 ODS2 COLUMN

See Table I for compound identification.

Compound No.	Intercept	Slope	r	d^*
1	1.276	-0.0220	-0.999	0.0094
2	1.737	-0.0270	-0.999	0.0094
3	1.322	-0.0235	-0.999	0.0137
4	2.900	-0.0400	-0.999	0.0197
5	1.868	-0.0288	-0.999	0.0138
6	2.279	-0.0325	-0.999	0.0143
7	1.847	-0.0288	-0.998	0.0206
8	3.428	-0.0452	-0.999	0.0214
9	1.498	-0.0252	-0.999	0.0089
10	1.956	-0.0299	-0.999	0.0128
11	1.490	-0.0256	-0.998	0.0216
12	3.109	-0.0426	-0.999	0.0255
13	3.241	-0.0432	-0.998	0.0290
14	3.684	-0.0476	-0.999	0.0249
15	3.262	-0.0439	-0.998	0.0286
16	4.728	-0.0584	-0.999	0.0208

* Mean distance of data points from the line.

TABLE III

PARAMETERS OF LOG k' VS. METHANOL CONTENT FOR μ BONDAPAK C₁₈ COLUMN

Other details as in Table II.

Compound No.	Intercept	Slope	r	d
1	1.167	-0.0173	-0.992	0.0279
2	1.605	-0.0223	-0.994	0.0309
3	1.186	-0.0180	-0.993	0.0289
4	2.785	-0.0364	-0.997	0.0377
5	1.749	-0.0243	-0.995	0.0311
6	2.192	-0.0293	-0.996	0.0357
7	1.724	-0.0244	-0.992	0.0404
8	3.660	-0.0476	-0.990	0.0889
9	1.473	-0.0220	-0.996	0.0374
10	1.876	-0.0260	-0.993	0.0388
11	1.398	-0.0209	-0.989	0.0426
12	3.041	-0.0398	-0.996	0.0471
13	3.156	-0.0405	-0.996	0.0445
14	3.678	-0.0466	-0.995	0.0630
15	3.155	-0.0410	-0.996	0.0507
16	4.738	-0.0580	-0.998	0.0452

TABLE IV

PARAMETERS OF $\log k'$ VS. METHANOL CONTENT FOR NUCLEOSIL 7C₆H₅ COLUMN

Other details as in Table II.

Compound No.	Intercept	Slope	r	d
1	0.975	-0.0138	-0.990	0.0253
2	1.250	-0.0173	-0.992	0.0289
3	1.011	-0.0144	-0.991	0.0259
4	2.272	-0.0300	-0.992	0.0501
5	1.334	-0.0185	-0.994	0.0272
6	1.623	-0.0220	-0.994	0.0325
7	1.359	-0.0188	-0.991	0.0328
8	2.705	-0.0354	-0.992	0.0595
9	1.215	-0.0170	-0.991	0.0297
10	1.510	-0.0207	-0.993	0.0320
11	1.255	-0.0175	-0.991	0.0306
12	2.492	-0.0325	-0.994	0.0457
13	2.607	-0.0341	-0.992	0.0564
14	2.894	-0.0375	-0.994	0.0535
15	2.611	-0.0341	-0.993	0.0534
16	3.828	-0.0480	-0.997	0.0447

$\log k'$ was calculated for compounds 1-16 at 40, 50, 60 and 70% methanol-water eluent mixtures and plotted against the methanol content of the eluent. The intercepts, slopes and correlation coefficients of the lines so obtained with each column are listed in Tables II-IV together with the mean distances of data points from the plots of $\log k'$ vs. methanol content.

Retention of isomeric derivatives

The lines of $\log k'$ vs. methanol content for the isomeric compounds (2 and 5, 3 and 9, 4 and 13, 7 and 10, 8 and 14, and 12 and 15, see Table I) are quite close to each other, revealing that the differences in retention are not large. A larger $\log k'$ value reflects greater retention, and in every case the line for the isomer having the larger group at the 4-phenyl position lies above the line of the 2-ethyl-substituted isomer. Hence, for monosubstituted compounds, 4-phenyl substitution results in greater retention than 2-ethyl substitution. Consistently, for disubstituted isomeric compounds, that having the larger group at the 4-phenyl position exhibits the greater retention. Of the compounds of general formula $R_1C_6H_4CONH(CH_2)_2R_2$, Nos. 10 ($R_1 = OCH_3$, $R_2 = CH_3$), 14 ($R_1 = C_6H_5$, $R_2 = CH_3$) and 15 ($R_1 = C_6H_5$, $R_2 = OCH_3$) have greater retention than, respectively, compounds 7 ($R_1 = CH_3$, $R_2 = OCH_3$), 8 ($R_1 = CH_3$, $R_2 = C_6H_5$) and 12 ($R_1 = OCH_3$, $R_2 = C_6H_5$).

Comparison of columns

The statistical analysis system (SAS) procedure RSQUARE was run to obtain correlations of $\log k'$ values on one column with $\log k'$ values on a second column at 40, 50, 60 and 70% methanol-water, i.e., $\log k'_1(40)$ vs. $\log k'_2(40)$, $\log k'_1(40)$ vs. $\log k'_2(50)$... $\log k'_1(70)$ vs. $\log k'_2(70)$, where the subscripts denote the different stationary phases and the percentage of methanol is given in parentheses. In this way

TABLE V

STATISTICAL DATA OBTAINED BY PLOTTING LOG k' VALUES ON ONE COLUMN AGAINST LOG k' VALUES ON A SECOND COLUMN AT 40, 50, 60 AND 70% METHANOL-WATER

Column pair	N	Correlation coefficient		
		Range	Average	Standard deviation
Spherisorb S5 ODS2- μ Bondapak C ₁₈	16	0.9945-0.9996	0.9977	0.0014
Spherisorb S5 ODS2-Nucleosil 7C ₆ H ₅	16	0.9824-0.9891	0.9891	0.0033
μ Bondapak C ₁₈ -Nucleosil 7C ₆ H ₅	16	0.9820-0.9941	0.9899	0.0034

sixteen correlations were obtained for each column pair. The statistical data for these correlations are presented in Table V.

The correlations at 50% methanol-water are

$$\log k'_1 = 1.096 \pm 0.009 - (0.127 \pm 0.008) \log k'_2 \quad r = 0.9996 \quad (1)$$

$$\log k'_1 = 1.432 \pm 0.059 - (0.198 \pm 0.042) \log k'_3 \quad r = 0.9884 \quad (2)$$

$$\log k'_2 = 1.306 \pm 0.052 - (0.065 \pm 0.040) \log k'_3 \quad r = 0.9891 \quad (3)$$

where the subscripts 1, 2 and 3 denote the data obtained on the Spherisorb S5 ODS2, μ Bondapak C₁₈ and Nucleosil 7C₆H₅ columns, respectively; the uncertainties are the standard errors of the regression coefficients. The results suggest that it is possible, even at different eluent compositions, to transfer a set of log k' values from one chromatographic system to another, and thus facilitate the prediction of log k' values from structural parameters on different stationary phases.

Comparison of log k' values with molecular connectivity indices

The molecular connectivity indices to the third order, ${}^0\chi$, ${}^0\chi^v$, ${}^1\chi$, ${}^1\chi^v$, ${}^2\chi$, ${}^2\chi^v$, ${}^3\chi_p$, ${}^3\chi_c$, ${}^3\chi_p^v$ and ${}^3\chi_c^v$, were calculated for compounds 1-16. The SAS procedure RSQUARE was used to obtain regressions of log k' against all possible one- and two-variable combinations of the connectivity level, χ , and the valence level, χ^v , indices, their reciprocals, squares and reciprocal squares. When the best combinations had been selected, procedure SYSREG was used to evaluate the regression coefficients.

The best one-variable fit was that using the second order valence level, ${}^2\chi^v$, on the two octadecyl phase columns and the first order valence level, ${}^1\chi^v$, on the phenyl phase column. The range of the regression coefficients ($N = 12$) was from 0.9531 to 0.9847 with a mean of 0.9701 and standard error of 0.0107.

The first order valence level, ${}^1\chi^v$, and the first order connectivity level, ${}^1\chi$, were chosen as variables for the best two-variable combinations in all except one case, where the zero order connectivity level, ${}^0\chi$, and the first order valence level, ${}^1\chi^v$, were chosen. The range of the regression coefficients ($N = 12$) was from 0.9911 to 0.9944 with a mean of 0.9933 and standard error of 0.0011. The third order indices were not chosen at all. The selected indices are given in Table VI.

TABLE VI

SELECTED MOLECULAR CONNECTIVITY INDICES FOR THE N-ETHYLBENZAMIDES

See Table I for compound identification.

Compound No.	${}^0\chi$	${}^1\chi^v$	${}^1\chi$	${}^2\chi^v$
1	8.1044	3.6755	5.3426	2.2255
2	8.8115	4.1755	5.8425	2.6219
3	9.5187	4.1653	6.3425	2.6147
4	11.9244	5.7326	8.3602	3.8575
5	8.9747	4.0862	5.7364	2.7255
6	9.6818	4.5862	6.2364	3.1219
7	10.3889	4.5760	6.7364	3.1147
8	12.7947	6.1433	8.7540	4.3575
9	9.6818	4.1985	6.2744	2.5880
10	10.3889	4.6985	6.7743	2.9844
11	11.0960	4.6883	7.2744	2.9772
12	13.5018	6.2557	9.2920	4.2200
13	12.0876	5.7468	8.3089	3.8028
14	12.7947	6.2468	8.8089	4.1993
15	13.5018	6.2367	9.3089	4.1921
16	15.9076	7.8040	11.3265	5.4349

The regression equations at 50% methanol-water with one- and two-variable combinations are:

Spherisorb S5 ODS2

$$\log k' = (0.544 \pm 0.039) {}^2\chi^v - 1.132 \pm 0.139 \quad r = 0.9645 \quad (4)$$

$$\log k' = (1.207 \pm 0.097) {}^1\chi^v - (0.550 \pm 0.066) {}^1\chi - 1.406 \pm 0.072 \quad r = 0.9935 \quad (5)$$

 μ Bondapak C₁₈

$$\log k' = (0.489 \pm 0.035) {}^2\chi^v - 0.920 \pm 0.122 \quad r = 0.9668 \quad (6)$$

$$\log k' = (1.074 \pm 0.086) {}^1\chi^v - (0.482 \pm 0.059) {}^1\chi - 1.173 \pm 0.064 \quad r = 0.9937 \quad (7)$$

Nucleosil 7C₆H₅

$$\log k' = (0.0260 \pm 0.001) ({}^1\chi^v)^2 - 0.098 \pm 0.040 \quad r = 0.9828 \quad (8)$$

$$\log k' = (0.051 \pm 0.008) ({}^1\chi^v)^2 - (0.012 \pm 0.004) ({}^1\chi^v) - 0.094 \pm 0.032 \quad r = 0.9914 \quad (9)$$

Choosing a connectivity level, χ , in a regression of this type implies that the nature of the atom itself is not important. If, however, a valence level, χ^v , is selected, the identity of the atom (carbon vs. oxygen) is important for the correlation.

CONCLUSIONS

A high degree of correlation was observed between the $\log k'$ on one column and the $\log k'$ on a second column, as well as between the molecular connectivity indices and the $\log k'$ at various eluent compositions on all three columns. The same connectivity parameters were chosen as the best descriptors of retention in these systems. These observations imply that in the range of alkylbenzamides examined the same structural features are important in the reversed-phase chromatographic process on these columns.

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