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## INVESTIGATION OF N-ALKYLBENZAMIDES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

### I. ISOCRATIC ELUTION CHARACTERISTICS OF THE C<sub>1</sub>-C<sub>5</sub> N-ALKYLBENZAMIDES

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#### SUMMARY

The reversed-phase liquid chromatographic retention of the C<sub>1</sub>-C<sub>5</sub> N-alkylbenzamides was investigated in aqueous-organic solvent mixtures. The log *k'* values for the amides were found to increase dramatically in mobile phases of low organic content. This non-linear relationship of log *k'* and mobile phase composition was observed on C<sub>18</sub> bonded-phase columns from different manufacturers. Attempts to measure retention data in pure water were successful only for the smaller molecules of the series. In general, the order of elution appears to parallel the carbon content of the N-alkyl group while increased branching decreases retention among isomeric amides.

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#### INTRODUCTION

The effect of mobile phase composition on retention is the topic of a great deal of current research<sup>1-4</sup>. Most experimental results published to date show linear plots of the variation of log capacity factor (*k'*) with solvent composition in reversed-phase liquid chromatography. Using a C<sub>18</sub> column, Karger *et al.*<sup>1</sup> demonstrated a linear plot for log *k'* versus solvent composition over the entire range of a methanol-water system for *n*-hexanol and *n*-octanol. But comparable plots for the same solutes in acetonitrile-water mixtures deviated from linearity at high water and high acetonitrile mobile phase compositions. Snyder and co-workers<sup>2,3</sup> have proposed that eqn. 1 is valid over a wide range of *k'* and solvent compositions:

$$\log k' = \log k'_w - S\phi_B \quad (1)$$

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The relationship produces a linear plot where  $k'_w$  is the capacity factor for the solute in pure water under isocratic conditions,  $\varphi_B$  is the volume fraction of organic solvent B in the mobile phase, and  $S$  is related to the strength of pure organic solvent B. They imply that the curvature observed by Schoenmakers *et al.*<sup>4</sup> in similar plots might be due to experimental error.

In a recent study on the chromatographic behavior of selected barbiturates, alkaloids, and substituted alkyluracils on a  $C_{18}$  bonded-phase column in methanol-water mixtures, Jandera *et al.*<sup>5</sup> stated that the observed deviations from linearity could be tolerated for plots of  $\log k'$  vs. solvent composition. They calculated a capacity factor for solutes in pure water by fitting a straight line to the experimentally measured data points. Tanaka and Thornton<sup>6</sup> reported plots of  $\log k'$  vs. mole fraction of methanol-water solvent mixtures for a homologous series of straight chain aliphatic monocarboxylic acids on a  $C_{18}$  column. The plots demonstrated a slight curvature, with the curves appearing parallel over the range  $0.2 \leq k' \leq 25$ . Extrapolations to pure water were not attempted. Schoenmakers *et al.*<sup>4</sup> studied the retention of a variety of aromatic solutes in mixtures of methanol, ethanol, and *n*-propanol in water on a  $C_{18}$  column. Their work suggested that a quadratic rather than a linear function of the eluent composition was a better fit of  $\log k'$  vs. solvent composition plots

$$\ln k' = A\varphi^2 + B\varphi + C \quad (2)$$

where  $\varphi$  is the volume fraction of the less polar eluent. In this equation, the coefficient  $C$  represents the retention value for pure water ( $\varphi = 0$ ). Schoenmakers reports that upon recalculation of some literature data<sup>1,7,8</sup>, quadratic dependencies can be demonstrated that were not recognized by the authors. But even with a quadratic function, some discrepancies can be seen in Schoenmakers' results. For example, values for  $\ln k'$  of 8.55, 7.70, and 4.53 in pure water for diethyl *o*-phthalate are predicted from data measured in methanol-, ethanol-, and propanol-water mixtures, respectively.

The work described here reports the reversed-phase chromatographic properties of the  $C_1$ - $C_5$  N-alkylbenzamides and attempts to describe the effects of eluent composition on retention.

## EXPERIMENTAL

### Apparatus

The liquid chromatograph consisted of a Waters Model 6000A solvent pump, Model U6K injector, Model 440 ultraviolet (UV) absorbance detector, an Alltech Associates high-performance liquid chromatographic (HPLC) column water jacket, and a Hitachi recorder. UV absorbance spectra were determined on a Hitachi 60 or a Perkin-Elmer Model 200 spectrophotometer. Infrared (IR) spectra were determined using a Beckman Acculab 6. Refractive index (RI) measurements were determined on an Erma refractometer. Constant temperature in both the HPLC column water jacket and the refractometer was maintained by a Haake Model FE constant temperature circulator.

### *Reagents and chemicals*

All chemicals were of reagent grade quality or better and were used as purchased without further purification. Methylamine was obtained from Matheson, Coleman & Bell (Norwood, OH, U.S.A.) and 2-methylbutylamine from Pfaltz & Bauer (Stamford, CT, U.S.A.). Spectrophotometric grade methanol was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.), spectrophotometric grade acetonitrile from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.), and spectrophotometric grade carbon tetrachloride from J. T. Baker (Phillipsburg, NJ, U.S.A.). Sodium nitrate was obtained from Allied Chemicals (Morristown, NJ, U.S.A.). All other chemicals were purchased from Aldrich (Milwaukee, WI, U.S.A.). Doubly distilled water was further purified by pumping (Waters Model 6000 solvent pump) through a column (7 cm × 2.1 mm I.D.) dry packed with Whatman CO:PELL ODS (30–38  $\mu\text{m}$ ) prior to preparation of solvent mixtures.

### *Synthesis of amides*

A solution of the appropriate primary amine (0.03–0.07 mole) in 35 ml of tetrahydrofuran was mixed with 200 ml of a 20% (w/v) solution of potassium carbonate in a three-necked flask equipped with a magnetic stirrer, reflux condenser, addition funnel, and a heating mantle. A solution of benzoyl chloride (two-fold molar excess) in 35 ml of tetrahydrofuran was added dropwise. The resulting mixture was heated at 50°C for 3 h. The mixture was maintained at or above pH 8 during the reaction. The solution was cooled to room temperature and extracted with chloroform (3 × 100 ml). The extract was dried with magnesium sulfate and evaporated. The resulting residue was purified by recrystallization from light petroleum (b.p. 30–60°C)–benzene or by column chromatography on silica gel (40 mesh) using a light petroleum–diethyl ether step gradient.

### *Mobile phase characteristics*

The mobile phase solvent mixtures were prepared and allowed to equilibrate for at least 1 h before use. The specific gravity and refractive index were measured for each batch of HPLC solvent mixture (methanol–water or acetonitrile–water) as a means of quality control. The specific gravity of the mobile phase was determined by completely filling a 25-ml Kimax specific gravity bottle equipped with a thermometer, side arm and a side tube cap with vent. The bottle and contents were weighed ( $\pm 0.1$  mg) at 25°C. The refractometer was calibrated to a value of 1.3325 at 25°C using the purified water. Refractive index values of the mobile phase solutions were determined at 25°C.

### *Chromatographic procedures*

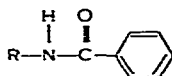
Two analytical HPLC columns were used in this study. A 25 cm × 4.60 mm I.D. Partisil ODS-2 (10  $\mu\text{m}$ ) column (1H3213) was purchased from Whatman (Clifton, NJ, U.S.A.), and a 15 cm × 4.60 mm I.D. Ultrasphere ODS (5  $\mu\text{m}$ ) column (UE795) was obtained from Altex Scientific (Berkeley, CA, U.S.A.). In operation, the columns were preceded by guard columns (7 cm × 2.1 mm I.D.) dry packed with Whatman CO:PELL ODS (30–38  $\mu\text{m}$ ). The guard column and analytical column were contained inside a column jacket and maintained at  $25.0 \pm 0.2^\circ\text{C}$  by circulating water from a constant temperature bath through the column jacket. The mobile

phase consisted of mixtures of water and methanol, or water and acetonitrile at a flow-rate of 1.5 ml/min. The column dead volume was taken as the elution volume for 0.5  $\mu$ l injection of an aqueous solution containing 0.3  $\mu$ g of sodium nitrate. The UV detector was operated at 254 nm and 0.005 a.u.f.s. Sufficiently dilute samples were prepared to give least detectable peaks (< 20% of scale). Capacity factors were calculated in the usual manner and based upon the average of at least two injections.

## RESULTS AND DISCUSSION

Reversed-phase liquid chromatography is a very effective tool for the separation of compounds differing only in the hydrocarbon portion of the molecule. In this report the isocratic elution characteristics of the C<sub>1</sub>–C<sub>5</sub> N-alkylbenzamides on C<sub>18</sub> bonded phases are evaluated. The amides were prepared from the appropriate primary amine and benzoyl chloride. The physical characteristics of these compounds are listed in Table I. The IR carbonyl absorption frequencies for the amides are very similar as are the values for the wavelength of maximum UV absorption indicating structural and electronic similarity in the "benzamide" portion of the molecules.

TABLE I  
PHYSICAL PROPERTIES OF BENZAMIDES



<i>R</i>		<i>m.p.</i> ( $^{\circ}$ C)	$\lambda_{max}$ (nm)	Carbonyl stretch ( $cm^{-1}$ )
Methyl	CH <sub>3</sub>	79– 82	225.5	1655
Ethyl	CH <sub>3</sub> CH <sub>2</sub>	68– 70	225.5	1652
<i>n</i> -Propyl	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	82– 84	225.5	1650
Isopropyl	(CH <sub>3</sub> ) <sub>2</sub> CH	98–103	225.2	1650
<i>n</i> -Butyl	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub>	oil	225.5	1652
Isobutyl	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	55– 58	225.2	1655
<i>sec.</i> -Butyl	CH <sub>3</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )	68– 78	225.2	1648
<i>tert.</i> -Butyl	(CH <sub>3</sub> ) <sub>3</sub> C	132–135	224.5	1658
<i>n</i> -Pentyl	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub>	31– 33	225.2	1650
<i>tert.</i> -Pentyl	CH <sub>3</sub> CH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub>	91– 93	224.0	1658
<i>sec.</i> -Pentyl	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )	73– 78	225.0	1650
Neopentyl	(CH <sub>3</sub> ) <sub>3</sub> CCH <sub>2</sub>	111–113	225.0	1658
Isopentyl	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> CH <sub>2</sub>	49– 53	225.5	1650
1-Ethylpropyl	(CH <sub>3</sub> CH <sub>2</sub> ) <sub>2</sub> CH	99–101	225.0	1652
1,2-Dimethylpropyl	(CH <sub>3</sub> ) <sub>2</sub> CHCH(CH <sub>3</sub> )	71– 75	225.0	1648
2-Methylbutyl	CH <sub>3</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub>	oil	225.0	1655
Cyclopentyl	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH	157–160	226.0	1650

The logarithms of the experimentally measured values of the capacity factors ( $k'$ ) for the amides at various mobile phase compositions are plotted in Fig. 1–4. The figures present data from two different C<sub>18</sub> columns using acetonitrile–water and methanol–water eluents. Retention data were collected at a minimum of six different

compositions of water-organic mobile phase for each compound. Solvent compositions were chosen to give a wide range of  $k'$  values for each solute. A  $\log k'$  of 2.0 or greater was achieved for most solutes as the maximum degree of retention. The  $\log k'$  values achieved on the Ultrasphere column (15 cm) are, in general, smaller than those attained on the Partisil column (25 cm). Equivalent  $\log k'$  values from the two columns represent a substantially longer analysis time for the longer column. For example a  $\log k'$  of 2.0 on Partisil ODS-2 requires 2.13 h of analysis time and 1.46 h on Ultrasphere ODS. The void volume is smaller on the Ultrasphere column and the plate count is greater (25,700 plates per meter for Partisil ODS-2 and 48,600 plates per meter for Ultrasphere ODS based on measurements made in this lab when the columns were new).

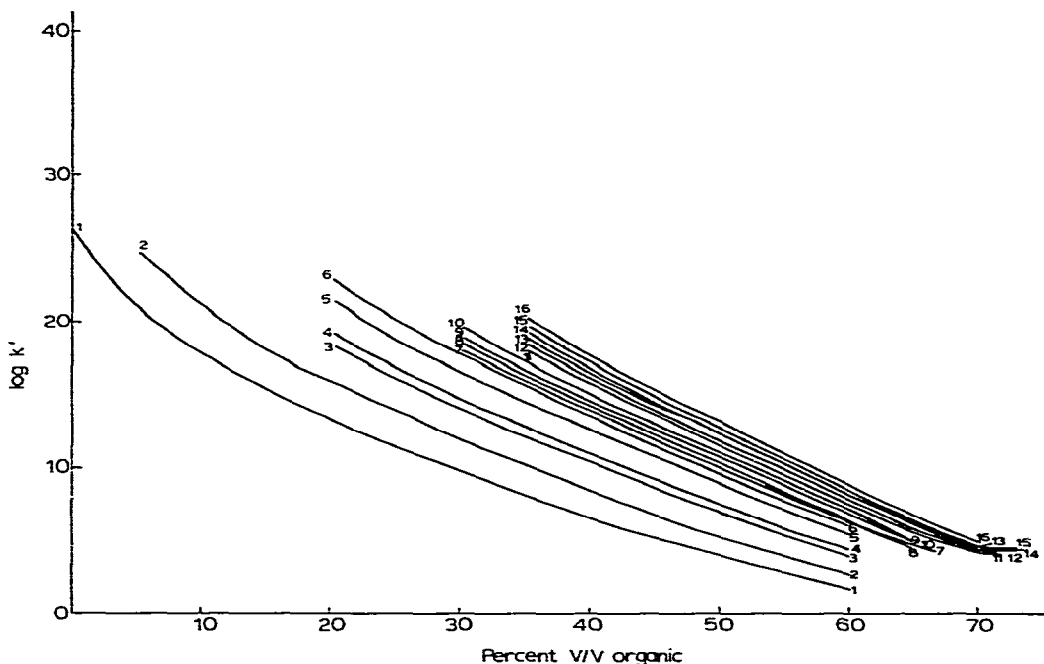


Fig. 1.  $\log k'$  versus solvent composition plots for N-alkylbenzamides on Partisil ODS-2 in methanol-water. Curves: 1, R = methyl; 2, R = ethyl; 3, R = isopropyl; 4, R = *n*-propyl; 5, R = *sec*-butyl; 6, R = isobutyl; 7, R = *tert*-butyl; 8, R = *n*-butyl; 9, R = 1-ethylpropyl; 10, R = 1,2-dimethylpropyl; 11, R = *sec*-pentyl; 12, R = neopentyl; 13, R = *tert*-pentyl; 14, R = 2-methylbutyl; 15, R = isopentyl; 16, R = *n*-pentyl. See general structure in Table I.

The effects of mobile phase composition on retention for the less retained members of the series are plotted in Figs. 5 and 6. Fig. 5 shows a plot of  $\log k'$  versus % (v/v) of the organic modifier for the compound N-methylbenzamide in acetonitrile and methanol-water solvent systems on the Partisil ODS-2 column. The open circles are for  $\log k'$  values which represent a  $k'$  less than 25. Plotting a straight line through the open circles in the form of eqn. 1 and extrapolation to a totally aqueous mobile phase, predicts the  $\log k'_w$  from acetonitrile-water data to be 1.605 ( $k'_w = 40.3$ ) with a correlation coefficient of  $-0.987$ . Similarly the  $\log k'_w$  is calculated to be 1.858 ( $k'_w =$

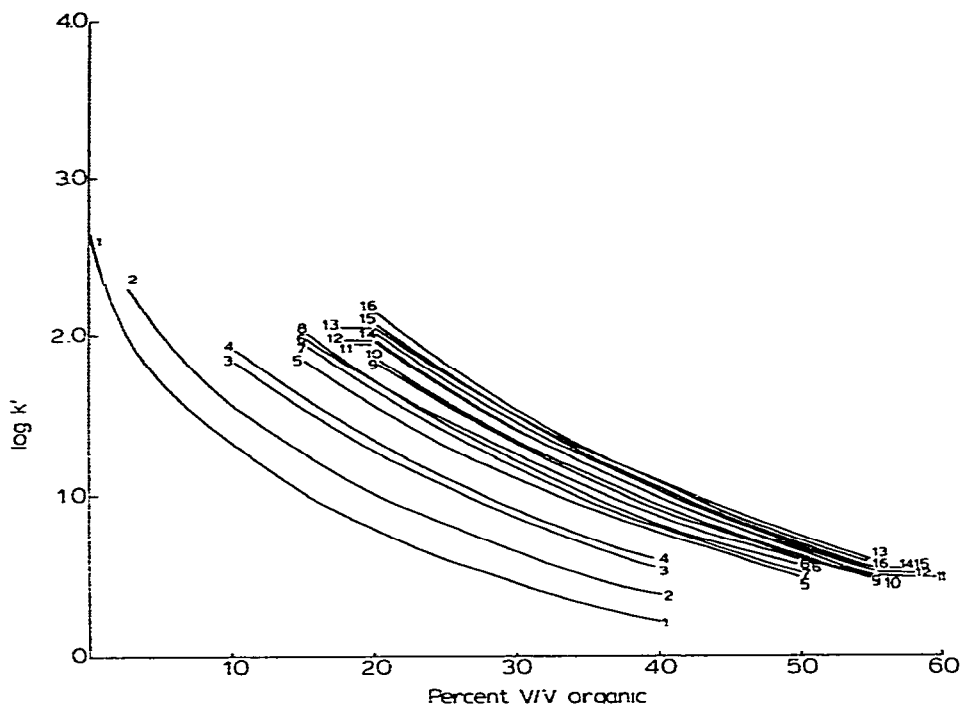


Fig. 2. Log  $k'$  versus solvent composition plots for N-alkylbenzamides on Partisil ODS-2 in acetonitrile-water. Curves: 1. R = methyl; 2. R = ethyl; 3. R = isopropyl; 4. R = *n*-propyl; 5. R = *sec*-butyl; 6. R = *tert*-butyl; 7. R = isobutyl; 8. R = *n*-butyl; 9. R = 1-ethylpropyl; 10. R = 1,2-dimethylpropyl; 11. R = neopentyl; 12. R = *sec*-pentyl; 13. R = *tert*-pentyl; 14. R = 2-methylbutyl; 15. R = isopentyl; 16. R = *n*-pentyl. See general structure in Table I.

72.0) with a correlation coefficient of  $-0.996$  from methanol-water data. These lines should intersect at 0% organic modifier (100% water), yet the error between the calculated intercepts is very significant in terms of the capacity factor difference predicted in this example. The line was only extrapolated over a span of 10% in acetonitrile-water and 20% in methanol-water. Furthermore, the predicted values are vastly different from the actual measured value of  $\log k'_w = 2.608$  ( $k'_w = 405.5$ ). It was not possible to directly measure  $k'_w$  values for any of the higher homologs on this column since a  $\log k'$  of 2.608 represents an elution time of 8.6 h.

The shorter elution times obtained on the Ultrasphere ODS column permitted the evaluation of three of the amides in a pure water mobile phase. The curvilinear relationship of  $\log k'$  and solvent composition was also observed for N-methylbenzamide on this column (Fig. 6). Furthermore, the retention data for the N-ethyl- and -isopropylbenzamides produced similar results. Fig. 6 shows a plot of the experimentally determined data for the three earliest eluting amides. An extrapolated linear plot of  $k' < 25$  data predicts a  $\log k'_w$  of 1.542 ( $k'_w = 34.8$ ) from the methanol-water data points,  $r = -0.994$ , and 1.404 ( $k'_w = 25.4$ ),  $r = -0.982$ , from the acetonitrile-water data for N-methylbenzamide. The measured value of  $\log k'_w$  for this compound was 1.957 ( $k'_w = 90.6$ ). In this example the line was extrapolated over a range of 10% in methanol-water and only 5% in acetonitrile-water. The measured capacity factors

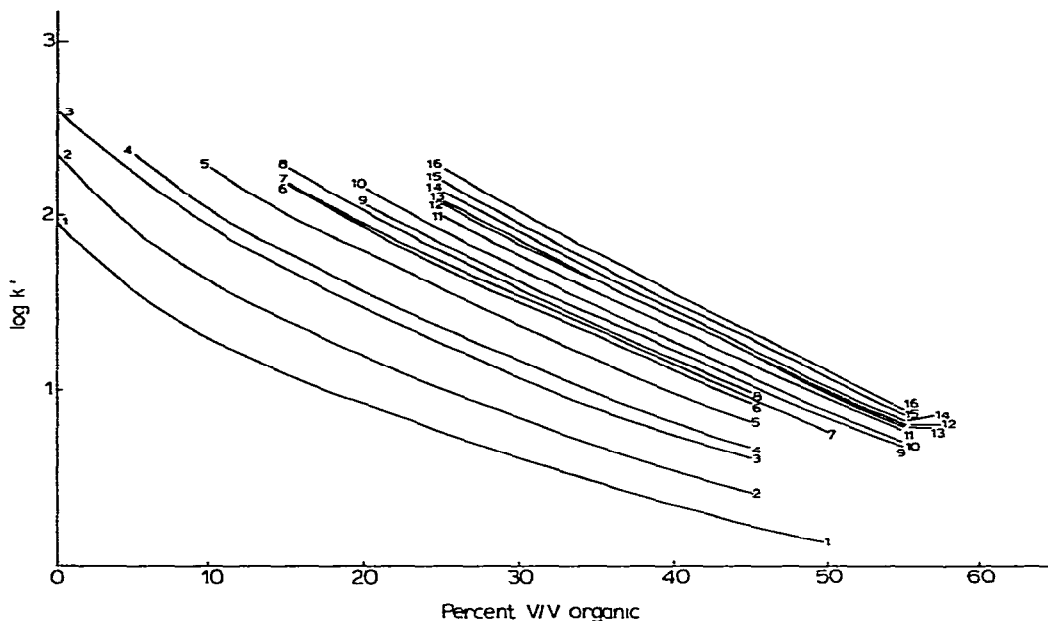


Fig. 3. Log  $k'$  versus solvent composition plots for N-alkylbenzamides on Ultrasphere ODS in methanol-water. Curves: 1. R = methyl; 2. R = ethyl; 3. R = isopropyl; 4. R = *n*-propyl; 5. R = *sec*-butyl; 6. R = isobutyl; 7. R = *tert*-butyl; 8. R = *n*-butyl; 9. R = 1-ethylpropyl; 10. R = 1,2-dimethylpropyl; 11. R = *sec*-pentyl; 12. R = *tert*-pentyl; 13. R = neopentyl; 14. R = 2-methylbutyl; 15. R = isopentyl; 16. R = *n*-pentyl. See general structure in Table I.

were observed to increase dramatically at low concentrations of organic modifier. This is particularly striking for N-methylbenzamide in acetonitrile-water (Table II). Linear extrapolation of the  $k' < 25$  data points for N-ethylbenzamide predicts a log  $k'_w$  of 1.862,  $r = -0.998$ , using the methanol-water data and 1.587,  $r = -0.987$  using the acetonitrile-water data. The measured data produced a log  $k'_w$  of 2.362 for N-ethylbenzamide. The same procedure predicted log  $k'_w = 2.092$ ,  $r = -0.998$  for N-isopropylbenzamide from the methanol-water data and 1.766,  $r = -0.993$ , from acetonitrile-water. Experimentally measured data gave a log  $k'_w$  of 2.601 for N-isopropylbenzamide.

Dolan *et al.*<sup>3</sup> outlined five points to consider when collecting data for the purpose of establishing the linearity or non-linearity of log  $k'$  vs. solvent composition plots. These are:

(a) complete equilibration of column and mobile phase before collecting data  
 (b) verification that  $k'$  is not a function of solute concentration, especially when  $k' < 5$

(c) constancy of the temperature of the column and incoming mobile phase during collection of  $k'$  data

(d) use of column packings that exhibit full coverage of the silica surface by the bonded phase

(e) determination of the possible error in  $t_0$  and its effect on reported  $k'$  values  
 These are valid points and have been taken into consideration while measuring the data presented here. All measurements were made using very dilute solutions which

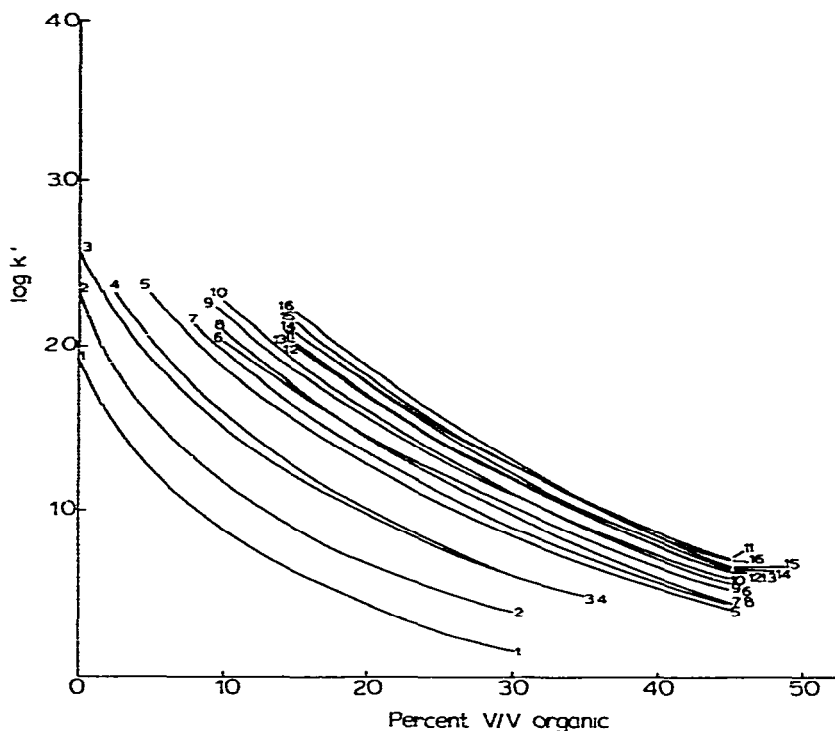


Fig. 4. Log  $k'$  versus solvent composition plots for N-alkylbenzamides on Ultrasphere ODS in acetonitrile-water. Curves: 1, R = methyl; 2, R = ethyl; 3, R = isopropyl; 4, R = *n*-propyl; 5, R = *sec*-butyl; 6, R = *tert*-butyl; 7, R = isobutyl; 8, R = *n*-butyl; 9, R = 1-ethylpropyl; 10, R = 1,2-dimethylpropyl; 11, R = *tert*-pentyl; 12, R = *sec*-pentyl; 13, R = neopentyl; 14, R = 2-methylbutyl; 15, R = isopentyl; 16, R = *n*-pentyl. See general structure in Table I.

gave peaks < 20% of full scale at 0.005 a.u.f.s. Because of the controversy surrounding the linearity vs. non-linearity of  $\log k'$  vs. organic modifier plots, the effect of concentration on the  $\log k'$  values measured at high concentrations of water in the mobile phase was investigated. On the Ultrasphere ODS column, the following percentage variations were found for N-methylbenzamide between the most dilute injection detectable and an injection representing 25 times that concentration: 0.9% variation at 10% methanol; 0.2% variation at 5% methanol; 0.1% variation at 2.5% acetonitrile; 2.2% variation at 0.5% acetonitrile (for 8.3 times the concentration); and 0.6% variation in pure water. If the values obtained at the higher concentration were plotted in Fig. 6 the difference made in the curve would hardly be noticeable. This information provides verification that the curvature observed in Fig. 5 and 6 does not result from concentration dependence. A measurement of the void volume was made for each chromatographic run using sodium nitrate without background electrolyte. This procedure actually determines the interstitial column volume<sup>9</sup> which is related to the true column void volume through a constant. Periodic measurements using sodium nitrate with background electrolyte (0.1 M phosphate buffer, pH 6.91) produced the true column void volume. The resulting void volume is higher than that measured without background electrolyte due to the electrochemical exclusion of



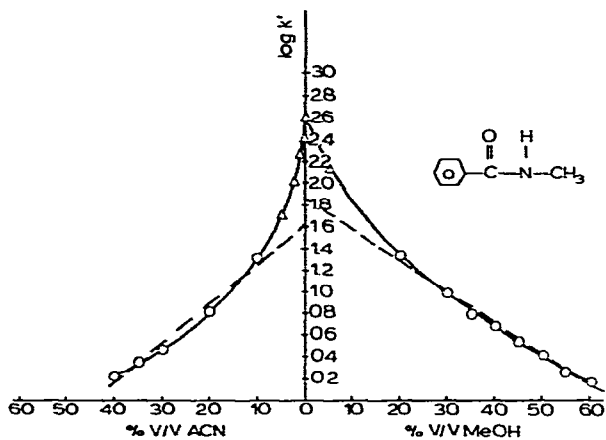


Fig. 5. Log  $k'$  versus solvent composition plots for N-methylbenzamide on Partisil ODS-2. ACN = Acetonitrile; MeOH = methanol.

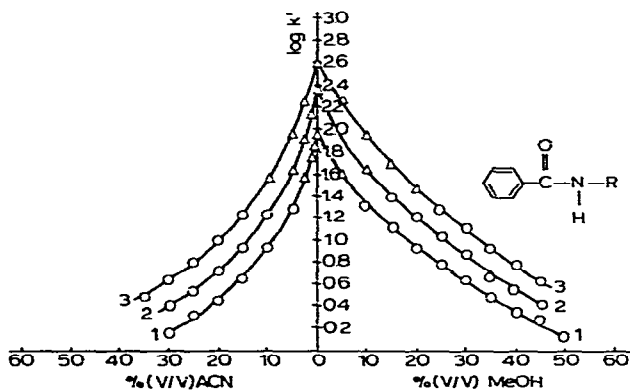


Fig. 6. Log  $k'$  versus solvent composition plots for N-alkylbenzamides on Ultrasphere ODS. Curves: 1, R = methyl; 2, R = ethyl; 3, R = isopropyl.

TABLE II

RETENTION DATA FOR N-METHYLBENZAMIDE IN LOW ACETONITRILE CONCENTRATIONS

Column	Acetonitrile (%, v/v)	$k'$	$\log k'$	Retention time (h)
Partisil ODS-2	1.0	178.2	2.251	3.8
	0.5	251.2	2.400	5.3
	0.0	405.5	2.608	8.6
Ultrasphere ODS	1.0	55.1	1.741	0.8
	0.5	70.3	1.847	1.0
	0.0	90.6	1.957	1.3

sodium nitrate from the pore structure of the silica particle in the absence of background electrolyte<sup>10</sup>. Using either method for measuring the column void volume produces no more than a 3% variation in calculated  $k'$  values in the non-linear portion of the  $\log k'$  vs. solvent composition plots and cannot account for the non-linearity of these plots. The similarities in the overall appearance of Figs. 5 and 6 is important to note. A linear model (eqn. 1) obviously does not suffice to explain the observed retention data, and efforts to improve this by using a quadratic model (eqn. 2) also proved futile.

The complete resolution of all sixteen benzamides studied was not obtained under isocratic conditions. The most efficient separation was obtained using the Ultrasphere ODS column with a methanol-water eluent (Fig. 7). Only peak 12 contained a mixture of two unresolved compounds, *N-tert.*-pentyl- and *N-neopentyl*benzamide. Solvent programming work was not attempted in this study. These results as well as Figs. 1-4 tend to point out relationships between structural features and elution order. In general, retention increases with the size of the *N*-alkyl group and chain branching reduces retention among isomers.

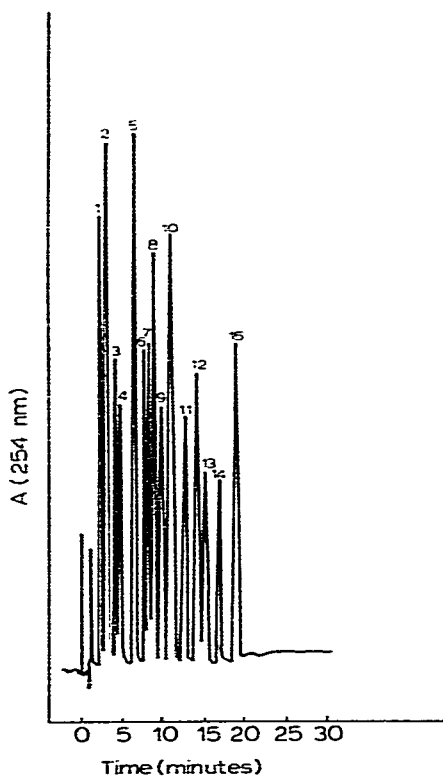


Fig. 7. Reversed-phase isocratic elution of the  $C_1$ - $C_5$  *N*-alkylbenzamides on Ultrasphere ODS in methanol-water (45:55). Peaks: 1, R = methyl; 2, R = ethyl; 3, R = isopropyl; 4, R = *n*-propyl; 5, R = *sec.*-butyl; 6, R = isobutyl; 7, R = *tert.*-butyl; 8, R = *n*-butyl; 9, R = 1-ethylpropyl; 10, R = 1,2-dimethylpropyl; 11, R = *sec.*-pentyl; 12, R = neopentyl and R = *tert.*-pentyl; 13, R = 2-methylbutyl; 14, R = isopentyl; 15, R = *n*-pentyl. See general structure in Table I.

## ACKNOWLEDGEMENT

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## REFERENCES

- 1 B. L. Karger, J. R. Grant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
- 2 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
- 3 J. W. Dolan, J. R. Gant and L. R. Snyder, *J. Chromatogr.*, 165 (1979) 31.
- 4 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
- 5 P. Jandera, J. Churáček and L. Svoboda, *J. Chromatogr.*, 174 (1979) 35.
- 6 N. Tanaka and E. Thornton, *J. Amer. Chem. Soc.*, 99 (1977) 7300.
- 7 R. M. Carlson, R. E. Carlson and H. L. Kopperman, *J. Chromatogr.*, 107 (1975) 219.
- 8 G. Vigh and J. Inczédy, *J. Chromatogr.*, 129 (1976) 81.
- 9 M. J. M. Wells and C. R. Clark, *Anal. Chem.*, 53 (1981) 1341.
- 10 G. E. Berendsen, P. J. Schoenmakers, L. de Galan, G. Vigh, Z. Varga-Puchony and J. Inczédy, *J. Liquid Chromatogr.*, 3 (1980) 1669.