

CHROM. 17 808

PROPERTIES OF SIX OCTADECYLSILANE STATIONARY PHASES FOR THE SEPARATION OF DANSYLAMIDES

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(Received April 4th, 1985)

SUMMARY

The properties of six different octadecylsilane (ODS) materials were examined for the separation of dansyl(Dns)-amides in acetonitrile-water, ethanol-water and methanol-water. The capacity factors, k' , of the solutes and solvent strengths, S , of the eluents are not related to the carbon content, % C (w/w), or the carbon content normalized with respect to the packing density, % C (w/ml), of the ODS phase. The solvent strength of ethanol and methanol is much higher than that of acetonitrile. In an homologous series the highest column selectivity is obtained for separating Dns-NH(CH₂CH₂CH₃) and Dns-NH(CH₂CH₃).

INTRODUCTION

Reversed-phase chromatography is the most commonly used technique for high-performance liquid chromatographic (HPLC) separations. This popularity is due to several factors including a high degree of reproducibility and its separation power. The main characteristics of the most commonly used octadecylsilane (ODS or RP-18) phases and their chromatographic properties have been examined by several workers¹⁻⁶. However, the discussion about the selectivity of ODS phases is inconsistent and sometimes contradictory.

Many suppliers produce phases labelled RP-18, but these can have vastly different chromatographic properties because of differences in the silica materials used as a support and the technique used to form the bonded phases.

Since reversed-phase packings show good retentivity towards non-polar species, compounds of this type are often chosen as test solutes. However, it is often the polar solutes that yield the most important information concerning the chemistry of reversed-phase materials.

In this paper the chromatographic properties of six ODS phases are evaluated using 5-dimethylaminonaphthalene-1-sulphonyl (Dns) amides as model compounds. Although it is mainly the hydrocarbon groups which interact with the bonded octadecylsilane groups, Dns-amides also contain basic amino groups which may interfere with unreacted and accessible silanol groups in the silica support, and thus cause additional effects on separations with different ODS phases.

TABLE I
COLUMNS USED IN THIS STUDY

Column Symbol	Material	Particle* size (μm)	Specific* surface area (m^2/g)	Pore* diameter (nm)	Packing** density (g/ml)	% C (w/ml)***	(w/w)**	Column dimensions (mm)	Supplier
BO	$\mu\text{Bondapak C}_{18}$	10	325	12.5	0.46	4.6	10	200 \times 4.6	Waters
HY	Hypersil ODS	5	170	12	0.69	6.9	9	200 \times 4.6	Shandon
LI	LiChrosorb RP-18	7	150	10	0.52	11.4	22	200 \times 4.6	Merck
NU	Nucleosil 5C ₁₈	5	350	10	0.53	8.3	15-16	200 \times 4.6	Macherey-Nagel
SP	Spherisorb S5 ODS2	5	220	8	0.74	8.8	12	200 \times 4.6	Phase Sep
UL	Ultrasphere ODS	5	—	8	0.83	10.0	12	150 \times 4.6	Alltech

* Data from manufacturer's specifications.

** Obtained by unpacking the column.

*** Normalized with respect to the packing density.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 1090 high-performance liquid chromatograph equipped with a HP 1040A diode-array detector, HP 85B personal computer, HP 3392A integrator and HP 9121 disc-drive unit for data storage were used.

Reagents and chemicals

5-Dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl), acetone, 1-propanol, 2-propanol, sodium nitrate and sodium bicarbonate were from Merck (Darmstadt, F.R.G.). All amines listed in Table II (except pentylamine and morpholine, which were from Merck) were from Fluka (Buchs, Switzerland). Ethanol was from Alko (Helsinki, Finland) and HPLC-grade methanol and acetonitrile were from Orion Corporation (Espoo, Finland). Water was distilled and deionized. All the reagents were pro analysis grade and were used without further purification.

Preparation of derivatives

A 100- μ mol amount of the amine was mixed in a test-tube with 5 ml of 60% (v/v) acetone in water containing 0.01 M sodium bicarbonate; 80 μ mol of Dns-Cl in acetone were then added. This mixture was stirred for 2 h at room temperature and stored in a refrigerator.

Columns

The columns used are listed in Table I. All columns except the Ultrasphere column (6 in Table I), which was commercial, were packed in the laboratory by a slurry technique using acetone as the suspending medium and a 50-ml slurry reservoir. The packing pressure was 450 bar.

Chromatographic procedures

The eluent was pumped isocratically at a flow-rate of 1 ml/min, and the oven temperature was 40°C. The elution volume for a 0.5- μ l injection of an aqueous solution containing sodium nitrate was taken as the column dead volume. Sufficiently dilute samples were prepared to give least detectable peaks at 250 nm (20% full scale).

RESULTS AND DISCUSSION

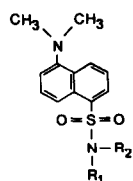
The retention of the compounds in Table II was measured on six different ODS columns in acetonitrile-water, ethanol-water and methanol-water. The solvent mixtures were selected so that the capacity factors, k' , would lie in the range 1–30. Four to eight measurements were made for each compound in each eluent mixture.

Variation of capacity factor with eluent composition

The analytical tool used to assess the selective effects of specific molecular structures is the variation of k' of the solute with the organic modifier content of the mobile phase

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

TABLE II
THE Dns DERIVATIVES INVESTIGATED



Compound No.	R ₁	R ₂
1	CH ₃	H
2	CH ₃ CH ₂	H
3	CH ₃ CH ₂ CH ₂	H
4	CH ₃ (CH ₂) ₂ CH ₂	H
5	CH ₃ (CH ₂) ₃ CH ₂	H
6	CH ₃ (CH ₂) ₄ CH ₂	H
7		
8		
9		
10	CH ₃	CH ₃
11	CH ₃ CH ₂	CH ₃ CH ₂
12	CH ₃	CH ₃ CH ₂ CH ₂ CH ₂
13	CH ₃	C(CH ₃) ₃
14	CH ₃ CH ₂	CH(CH ₃) ₂
15	CH ₃ CH ₂ CH ₂	CH ₃ CH ₂ CH ₂
16	CH ₃ CH ₂ CH ₂ CH ₂	CH ₃ CH ₂ CH ₂ CH ₂
17	CH ₃	

where φ is the volume fraction of organic solvent in the water-organic solvent mixture, k'_w represents the capacity factor of a solute with pure water as mobile phase and S , the slope of the regression curve, should be related to the solvent strength of the pure organic solvent^{7,8}. Larger values of S lead to a faster decrease in k' with increasing φ .

Dolan *et al.*⁹ reported that S depends on (i) the deviation of $\log k'$ versus φ from linearity, (ii) change in solute structure, (iii) type of reversed-phase packing and (iv) type of eluent. The variation of S with solute structure is evident also from our earlier study on Dns-amides¹⁰.

In this study the regression coefficient, r , of the plots of $\log k'$ versus φ varied between 0.968 and 0.999. The mean S values on different reversed-phase packings and solvents are presented in Fig. 1. The lowest S values are obtained for acetonitrile, with much higher and roughly equal S values for ethanol and methanol. This means that changes in ethanol or methanol content cause a much larger change in retention than do changes in acetonitrile content. In ethanol-water an especially dramatic effect is obtained on LiChrosorb C₁₈ (Fig. 1).

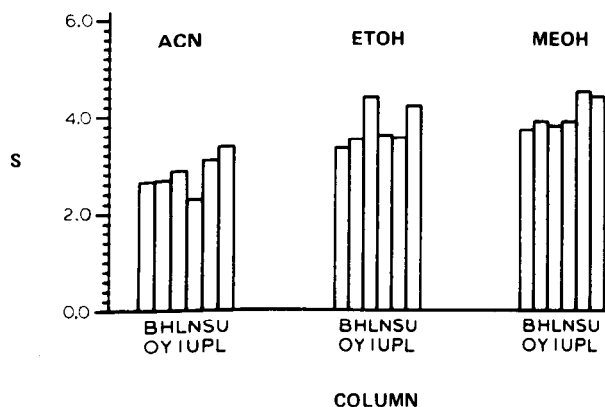


Fig. 1. Mean values of solvent strength obtained using seventeen Dns-amides as solutes on different columns in acetonitrile-water (ACN), ethanol-water (ETOH) and methanol-water (MEOH). See Table I for column identification.

Regression analysis on S and the carbon content of the ODS phase (Table I) gives only a poor correlation: $r = 0.25$ in acetonitrile-water, 0.21 in ethanol-water and 0.39 in methanol-water. A slightly better correlation is obtained by using the carbon content normalized with respect to the packing density (Table I): $r = 0.45$, 0.89 and 0.38, respectively. The poor correlation implies that the variations in S are not related to the carbon content of the packings material. The presence of free silanol groups in the silica support may be important in explaining this behaviour.

Capacity factors on different ODS materials

Engelhardt and Ahr¹ stated that the properties of chemically bonded phases depend on several factors such as the silica itself, its physical properties (specific surface area, pore diameter and packing density), surface concentration of the bonded alkyl groups, etc. In order to characterize the octadecylsilane stationary phases, retention data measured at representative water-organic solvent compositions, e.g., 66% (v/v) acetonitrile, 52% (v/v) ethanol and 70% (v/v) methanol in water, were

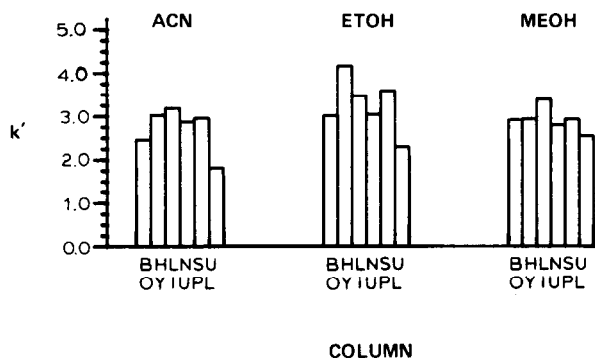


Fig. 2. Experimental k' values of compound 3 in 66% (v/v) acetonitrile in water (ACN), in 52% (v/v) ethanol in water (ETOH) and 70% (v/v) methanol in water (MEOH). See Table I for column identification.

TABLE III

EXPERIMENTAL k' VALUES IN 66% ACETONITRILE IN WATER

See Table I for column and Table II for compound identification.

Compound No.	Column					
	BO	HY	LI	NU	SP	UL
1	1.63	1.90	2.01	1.72	1.74	1.00
2	1.97	2.38	2.50	2.19	2.24	1.32
3	2.46	3.03	3.19	2.88	2.96	1.82
4	3.13	3.97	4.16	3.86	3.99	2.72
5	4.03	5.33	5.56	5.27	5.49	3.88
6	5.29	7.33	7.61	7.35	7.63	5.51
7	3.12	4.08	4.21	3.95	4.07	2.67
8	4.30	5.92	6.10	5.94	6.10	4.37
9	2.12	2.68	2.92	2.58	2.70	1.77
10	2.50	3.11	3.26	3.10	3.06	1.91
11	3.65	4.82	4.99	4.99	4.91	3.43
12	5.12	6.95	7.33	7.32	7.41	5.38
13	4.37	6.14	6.13	6.12	6.13	4.42
14	4.47	6.16	6.30	6.33	6.33	4.55
15	6.21	9.08	9.16	9.58	9.44	7.02
16	10.6	17.2	17.1	18.7	18.3	14.1
17	5.21	7.13	7.33	7.25	7.33	5.32

TABLE IV

EXPERIMENTAL k' VALUES IN 52% ETHANOL IN WATER

See Table I for column and Table II for compound identification.

Compound No.	Column					
	BO	HY	LI	NU	SP	UL
1	1.74	2.31	1.92	1.56	1.75	1.03
2	2.22	3.01	2.50	2.24	2.36	1.50
3	3.02	4.18	3.48	3.05	3.60	2.30
4	4.28	6.02	5.09	4.59	5.80	3.64
5	6.11	8.80	7.58	7.02	8.50	5.75
6	8.93	13.0	11.4	10.8	14.8	9.09
7	3.56	5.23	4.13	3.98	4.50	2.86
8	5.46	8.33	6.72	6.35	8.40	5.04
9	2.38	3.39	2.68	2.36	2.60	1.66
10	2.64	3.78	3.01	2.68	3.20	1.91
11	4.38	6.50	5.23	4.91	6.20	3.80
12	7.10	10.9	8.98	8.65	11.8	7.03
13	5.64	8.50	6.96	6.53	8.50	5.30
14	5.79	8.70	7.12	6.89	9.00	5.47
15	9.18	14.4	11.9	11.7	15.0	9.70
16	19.6	31.6	29.5	27.9	29.0	23.9
17	7.82	12.7	10.2	9.78	12.8	7.97

TABLE V

EXPERIMENTAL k' VALUES IN 70% METHANOL IN WATER

See Table I for column and Table II for compound identification.

Compound No.	Column					
	BO	HY	LI	NU	SP	UL
1	1.77	1.65	2.20	1.56	1.55	1.22
2	2.23	2.15	2.60	2.18	2.09	1.80
3	2.92	2.94	3.40	2.80	2.93	2.55
4	3.95	4.22	4.70	4.35	4.34	3.95
5	5.47	6.28	7.40	6.30	6.49	6.00
6	7.81	9.56	10.7	10.0	9.93	8.70
7	3.74	3.74	4.40	3.00	4.09	3.60
8	5.47	5.93	6.80	6.40	6.69	5.80
9	2.60	2.61	3.20	2.70	2.74	2.30
10	2.76	2.63	3.25	2.75	2.85	2.35
11	4.24	4.45	5.20	4.80	4.85	4.30
12	6.54	7.47	8.30	7.90	8.26	7.70
13	5.36	5.90	6.80	6.30	6.47	5.70
14	5.46	6.08	7.00	6.50	6.67	6.20
15	8.26	10.2	11.0	10.6	11.0	10.0
16	16.7	22.4	27.0	26.0	25.6	25.0
17	7.36	8.37	9.50	9.50	9.30	8.60

selected from the whole retention data for a closer study (Tables III–V). Capacity factors for compound 3 in different eluents on Nucleosil 5C₁₈ are given in Fig. 2.

Engelhardt and Ahr¹ reported that the capacity factors obtained on different stationary phases are not correlated with the differences in carbon content; but a much better correlation is obtained if the carbon content is normalized with respect to the packing density of the stationary phase and the amount of carbon per unit column volume. In our measurements, neither % C nor normalized % C gave high correlations with $\log k'$ (Table VI). The difference in the results is probably due to the solutes used. They used hydrocarbons, which are more affected by the bonded carbon groups than are our more polar Dns-amides.

In each eluent the lowest retention is obtained on Ultrasphere ODS. The largest retention obtained is dependent on the eluent type. LiChrosorb RP-18 in

TABLE VI

CORRELATION COEFFICIENTS OBTAINED FOR REGRESSION ANALYSIS OF $\log k'$ (COMPOUND 3) vs. CARBON CONTENT, % C (w/w), AND THE CARBON CONTENT NORMALIZED WITH RESPECT TO THE PACKING DENSITY, % C (w/ml)

Eluent	Correlation coefficient	
	$\log k'$ vs. % C (w/w)	$\log k'$ vs. % C (w/ml)
66% Acetonitrile	0.402	0.037
52% Ethanol	0.045	0.155
70% Methanol	0.642	0.217

acetonitrile–water and methanol–water and Hypersil ODS in ethanol–water give the largest retention (Fig. 2). LiChrosorb RP-18 has the highest % C (w/ml) and μ Bondapak C₁₈ the lowest (Table I).

Some compounds are difficult to separate from each other. Compounds 5, 8, 13 and 14, which all contain five carbon atoms in the amino part, are not well separated on any of these columns. On the other hand, compound 12, which also contains five carbon atoms, is easily separated from these compounds. In reversed-phase chromatography, crowded hydrocarbon groups cause shorter retention among isomeric compounds. Compound 5 is not crowded at all, but it is the only one of these compounds that is formed from a primary amine and thus contains a polar N–H bond.

Another group of compounds that are not easily separated from each other (especially in methanol–water) comprises compounds 3, 9 and 10.

Compound 4, 7 and 11 behave quite differently in the different eluent types. Column selectivities, $\alpha = k'(2)/k'(1)$, for compounds 4 and 7 are presented in Fig. 3. These compounds are difficult to separate from each other in acetonitrile–water and in methanol–water, but can quite easily be separated in ethanol–water, as the high α values indicate (Fig. 3). On the other hand, compounds 4 and 11 are easily separated in acetonitrile–water, but very poorly in ethanol–water.

Column selectivity in an homologous series

Compounds 1–6 form an homologous series. The α values for a methylene-group increment were calculated in different eluents. The results in acetonitrile–water are presented in Fig. 4. The highest selectivity is obtained for separating compounds 2 and 3. The selectivities for the remainder are appropriately equal. Only slight differences are obtained with different columns.

Gradient elution

Gradient elution was performed from 40% (v/v) acetonitrile in water to 80%

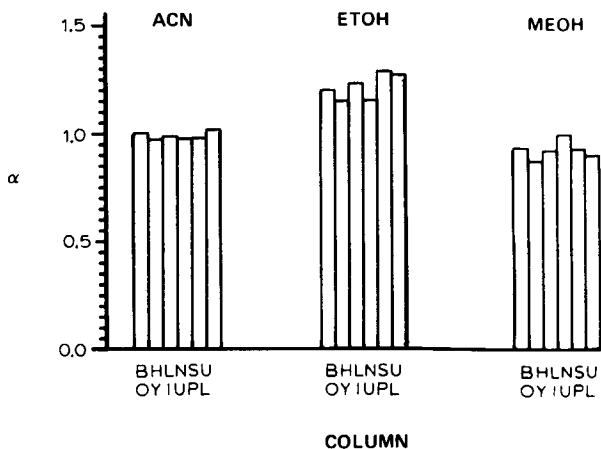


Fig. 3. Column selectivities, α , for separating compounds 4 and 7 in 66% (v/v) acetonitrile in water (ACN), in 52% ethanol in water (ETOH) and 70% (v/v) methanol in water (MEOH). See Table I for column identification.

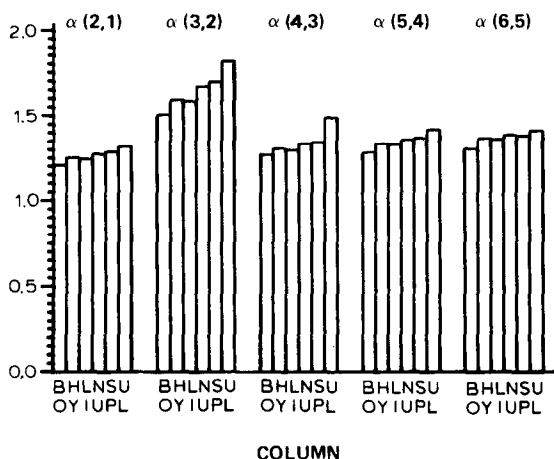


Fig. 4. Column selectivities, α , for compounds in an homologous series in 66% acetonitrile in water. The compound numbers are given in parentheses. See Table I for column identification and Table II for compound identification.

TABLE VII
EXPERIMENTAL k' VALUES IN GRADIENT ELUTION FROM 40% ACETONITRILE IN WATER TO 80% IN 40 MIN

See Table I for column identification and Table II for compound identification.

Compound No.	Column					
	BO	HY	LI	NU	SP	UL
1	5.48	6.03	6.79	6.58	6.62	4.89
2	7.04	8.01	8.78	8.45	8.80	6.77
3	9.04	10.5	11.3	10.8	11.6	9.25
4	11.2	13.4	14.1	13.3	14.6	12.0
5	13.5	16.3	16.9	15.8	17.7	15.0
6	15.8	19.4	19.8	18.4	20.9	18.1
7	10.6	12.5	13.4	12.9	14.0	11.3
8	13.4	16.3	17.1	16.2	18.0	15.1
9	7.91	8.92	9.91	9.70	10.1	7.80
10	8.70	9.91	11.0	10.6	11.3	8.75
11	12.1	14.4	15.3	14.6	16.2	13.2
12	15.2	18.4	19.1	18.1	20.4	17.3
13	13.7	16.4	17.3	16.3	18.4	15.3
14	13.9	16.8	17.5	16.6	18.7	15.7
15	16.8	20.6	21.2	19.9	22.7	19.5
16	21.2	26.4	26.7	24.9	28.8	25.3
17	15.7	18.6	19.4	18.5	20.6	17.6

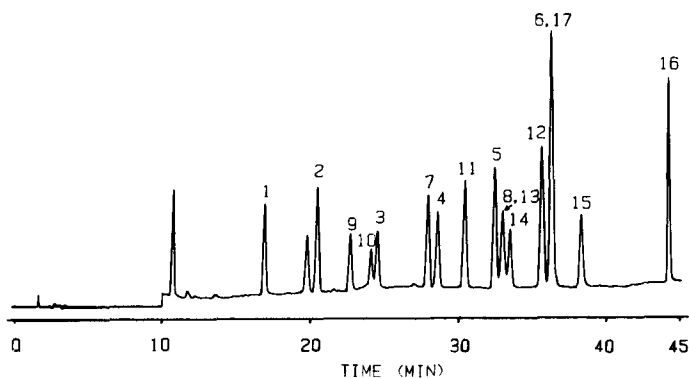


Fig. 5. Optimized separation of compounds 1–17 on Nucleosil 5C₁₈ column. The gradient elution used was from 30% (v/v) acetonitrile in water to 66% in 36 min and then to 90% in the following 9 min. For compound identification see Table II.

water in 40 min on each of the columns. The capacity factors measured are given in Table VII. The elution order is the same in isocratic and gradient elution. An optimized gradient elution on the Nucleosil 5C₁₈ column is illustrated in Fig. 5. Gradient elution does not solve the problem of separating compounds 5, 8, 13 and 14 from each other. On the other hand, compounds 4, 7 and 11 are now well separated.

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