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RETENTION BEHAVIOR OF LONG CHAIN QUATERNARY AMMONIUM HOMOLOGUES AND RELATED NITROSO-ALKYLMETHYLAMINES

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SUMMARY

Several chromatographic methods have been utilized to study the retention behavior of a homologous series of *n*-alkylbenzyldimethylammonium chlorides (ABDAC) and the corresponding nitroso-*n*-alkylmethylamines (NAMA). Linear correlation of the logarithmic capacity factor (k') with the number of carbons in the alkyl chain provides useful information on both gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) retention parameters of unknown components. Under all conditions employed, GC methodology has proved effective in achieving complete resolution of the homologous mixture of NAMA despite its obvious inadequacy in the separation of *E–Z* configurational isomers. Conversely, normal-phase HPLC on silica demonstrates that the selectivity (α) value for an *E–Z* pair is much higher than that for an adjacent homologous pair. In the reversed-phase HPLC study, three different silica-based column systems were examined under various mobile phase conditions. The extent of variation in k' was found to be a function of the organic modifier, counter-ion concentration, eluent pH, nature of counter-ion, and the polarity and type of stationary phase. The k'–[$NaClO_4$] profiles showed similar trends between the ABDAC and the NAMA series, supporting the dipolar electronic structures of the latter compounds. Mobile phase and stationary phase effects on component separation are described. The methodology presented establishes the utility of HPLC separation techniques as versatile analytical tools for practical application.

INTRODUCTION

The manufacture of quaternary ammonium compounds by surfactant industries often produces, among other amine products, mixtures of homologous long chain alkylbenzyldimethylammonium halides (C_8–C_{18}). These compounds represent an important group of commercial chemicals used extensively as additives in cosmetics, lubricants, paints, soil stabilizers, and surface-active materials. The title high-molecular-weight quaternary amines are relatively non-toxic and generally possess antibacterial properties. They constitute the active ingredients in a large number of

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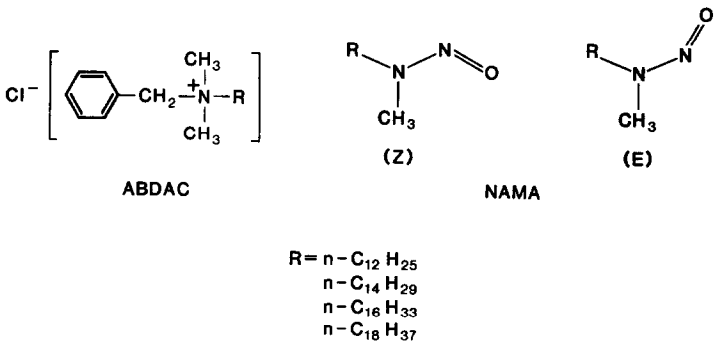

Fig. 1. Structures of alkylbenzyldimethylammonium chlorides (ABDAC) and nitroso-alkylmethylamines (NAMA). The (*E*) and (*Z*) denote configurational isomers.

domestic health-care products, veterinary therapeutants, and popular disinfectants for water treatment. In fish culture, bactericidal formulations of quaternary ammonium salts have been routinely used for treatment of bacterial gill disease. The mixtures commercially formulated for this purpose typically contain *n*-alkyl homologues of alkylbenzyldimethylammonium chlorides (ABDAC) in which the number of carbons on the long chain *n*-alkyl group extends from 12 to 18 (Fig. 1).

Interest in the development of new analytical methods for trace analysis of quaternary ammonium compounds continues unabated. Since the initial report[1] describing a gas chromatographic (GC) technique for the determination of ABDAC mixtures via the cyanamide and trichloroethyl carbamate derivatives, we have taken two additional approaches to the development of sensitive and specific detection methods: (1) fast atom bombardment mass spectrometry (MS) with stable isotope internal standardization[2], and (2) combined GC–thermal energy analysis (TEA) and combined high-performance liquid chromatography (HPLC)–TEA of *n*-alkylmethylamine homologues (NAMA) obtained by two-step chemical derivatization of the parent ABDAC salts[3]. The utility of deploying these techniques has been demonstrated. During the course of the GC and HPLC investigations, it was necessary to carry out numerous exploratory experiments to effect chromatographic optimization and to delineate chromatographic characteristics of relevant components. In the present paper, we describe these studies with principal emphasis on the retention behavior of the two homologous series of compounds denoted by ABDAC and NAMA (Fig. 1). Results are discussed with regard to the nature of the influence of chromatographic variables derived from both mobile and stationary phases upon capacity factor (k'), resolution (R), and selectivity (α) parameters. Additionally, some aspects of configurational preferences of the NAMA series are reported.

EXPERIMENTAL

Chemicals and reagents

Pure octyl-(8-ABDAC), dodecyl-(12-ABDAC), tetradecyl-(14-ABDAC), hexadecyl-(16-ABDAC), and octadecylbenzyldimethylammonium chlorides (18-ABDAC) were synthesized at the National Fishery Research Laboratory (La Crosse, WI, U.S.A.) by procedures[1] developed previously at this laboratory. Pure octyl-(8-

NAMA), dodecyl-(12-NAMA), tetradecyl-(14-NAMA), hexadecyl-(16-NAMA), and octadecylmethylnitrosamines (18-NAMA) were prepared from the corresponding *n*-alkyldimethylamines (Armak, Chicago, IL, U.S.A.) and sodium nitrite according to a standard method for similar compounds[4]. The crude NAMA products were purified twice by preparative thin-layer chromatography (TLC) [Analtech Uniplate silica gel GHLF (0.1 × 20 × 20 cm); solvent, benzene–chloroform–methanol–acetic acid (8:5:3:1)]. All chromatographic solvents were reagent grade obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Sodium methane sulfonate was prepared by treating the relevant sulfonic acid with sodium methoxide in methanol followed by recrystallization from acetonitrile–methanol (5:1). Other members of higher homologues of sodium alkyl sulfonates, including dimethylcyclohexane sulfonate, were purchased from Aldrich (Milwaukee, WI, U.S.A.) and were used without further purification. With the exception of sodium perchlorate (Alpha Products, Danvers, MA, U.S.A.), inorganic acids and salts were supplied by J. T. Baker. All reagents used in this work were of high purity and were used as received from commercial sources.

GC and HPLC

Unless stated otherwise, GC data were generated by using a Varian Model 3711 gas chromatograph equipped with a flame ionization detector. For comparative studies on the GC retention behavior of NAMA compounds, coiled glass columns [100 cm (or 200 cm) × 6.35 mm O.D., 2 mm I.D.] packed with the following stationary phases were used: (a) 5% OV-101 on Gas-Chrom Q (80–100 mesh), (b) 5% Carbowax 20M on Gas-Chrom Q (80–90 mesh), (c) 5% OV-1 on Gas-Chrom Q (80–100 mesh), (d) 3% SP-2250 DB on Supelcoport (100–120 mesh), (e) 10% Carbowax 20M on Chromosorb W AW (80–100 mesh), (f) 10% Carbowax 20M–2% potassium hydroxide on Chromosorb W AW (80–100 mesh), (f′) 5% Carbowax 20M on Supelcoport (100–120 mesh), (f″) 10% Carbowax 20M–0.5% potassium hydroxide on Chromosorb W HP (80–100 mesh), and (g) 10% UC-W982 on Gas-Chrom Q (80–100 mesh). These alphabetical designations for various stationary phases correspond to those for the correlation lines shown in Fig. 2. For normal GC operation, the instrument was functioned isothermally within the optimal temperature range of 180–220°C depending on the length of the column, while the carrier gas (nitrogen) was passed through the column at a flow-rate of 30 ml/min. Detector and injector temperatures were set at 360°C and 250°C, respectively. Chromatograms were recorded on a Beckman Model 1005 recorder and retention times were measured automatically with an electronic digital data system (Varian Model CDS 111C).

In HPLC studies, a Varian Model LC-5020 liquid chromatograph interfaced with a variable-wavelength UV–Vis detector (Varian Varichrom) and a Varian Model 19176 strip chart recorder was used. Column effluents were monitored with the absorbance detector set at 240 nm for nitroso-compounds in the NAMA series and at 215 nm for quaternary amines in the ABDAC series. Retention measurements were performed on a Varian Model CDS111L data system. In a typical HPLC analysis, samples containing 10–50 μg/ml of individual analytes were injected into an analytical column via an injector port [Valco injector valve (CV-6-UHPa-N60) and 10-μl loop] and a guard column (5 cm × 4 mm I.D.). The guard column was packed with a pellicular material of 40 μm average particle size matching the stationary phase ma-

terial in the analytical column. To ensure accurate peak measurements for highly retentive components, the sample concentration was increased to provide reasonable peak height during a chromatographic assay. Several different, microparticulate silica-based HPLC columns were obtained from industrial suppliers. The chemically bonded silica columns employed for the reversed-phase HPLC studies include Varian MicroPak MCH-10 (10 μm, 30 cm × 4 mm I.D.), Analytical International (Harbor City, CA, U.S.A.), Sepralyte C 18 and Sepralyte PH (both columns, 5 μm, 25 cm × 4.6 mm I.D.), and Altex (Berkeley, CA, U.S.A.), Ultrasphere ODS and Ultrasphere Cyano (both columns, 5 μm, 25 cm × 4.6 mm I.D.). Normal-phase HPLC analyses were carried out separately on Altex Ultrasphere Si and Ultrasphere Cyano columns (5 μm, 25 cm × 4.6 mm I.D.). Details of specific HPLC conditions for various experiments are given in the Results and Discussion section. In general, reversed-phase solvent systems consisted of water and an organic modifier for the analysis of non-ionic NAMA compounds. Three organic modifiers were employed in this study: acetonitrile, methanol and tetrahydrofuran (THF).The presence of some counter ions in the "water–organic modifier" mobile phase was critical to facilitate elution of the ionic ABDAC compounds through reversed-phase columns. Mobile phases containing variable amounts of water and counter ion salts (0.005–0.5 *M*) over a pH range of 2–6 were prepared with high precision to suit various experimental requirements.

Buffer solutions were prepared by adding either phosphoric acid (or perchloric acid) or a dilute solution of sodium hydroxide to 0.01 *M* sodium dihydrogenphosphate solutions until the pH meter indicated the desired pH readings. The ionic strength of all solutions was kept constant throughout the same set of experiments where a fluctuation in ionic strength might affect the chromatographic results. Typical mobile phases employed in the present normal-phase HPLC study were composed of hexane, methylene chloride, and a small amount of isopropanol (ISP). Frequently THF was added to the mobile phase to improve the normal-phase separation of the nitroso-compounds[5]. Unless otherwise specified, mobile phase eluents were pumped through the column invariably under isocratic conditions at a flow-rate of 2 ml/min and at ambient temperature. Retention and resolution parameters were determined by conventional methods based on the void volume and band width measurements.

Preparative HPLC for the isolation of E and Z isomers of NAMA homologues

To isolate a pure configurational isomer from the mixture of *E–Z* isomers inherent with each NAMA homologue for use in its structural identification, samples (20–50 mg) of 12-NAMA, 14-NAMA, 16-NAMA, and 18-NAMA obtained from preparative TLC were individually chromatographed on an Altex Ultrasphere ODS column (5 μm, 25 cm × 10 mm I.D.) with mobile phases containing different proportions of methanol and water at a flow-rate of 0.5 ml/min. Ratios of methanol to water in the mobile phases for the separation of the lower members (12-NAMA and 14-NAMA) and the higher members (16-NAMA and 18-NAMA) of the homologous series were 17:3 and 9:1, respectively. Column eluates were collected into 0.5-ml fractions with a Buchler linear automatic fraction collector. A Varian refractive index detector was interfaced with the preparative HPLC system to monitor the fractionation of the *E* and *Z* components. Aliquots of combined fractions that apparently

contained a single isomer were further analyzed by normal-phase HPLC on an analytical silica column (Altex Ultraspher Si, 5 μm, 25 cm × 4.6 mm I.D.) with UV detection at 240 nm. The mobile phase for this normal-phase separation was 97:3 hexane–[methylene chloride–THF–ISP (1:1:0.1)]. A pure isomer (*E* or *Z*) was then isolated by evaporation of homogeneous fractions and extraction with methylene chloride followed by exhaustive drying under reduced pressure. A sample of this material was subjected to structural determination by GC-chemical ionization MS [$(M^+ + 1)$:12-NAMA, *m/e* 229; 14-NAMA, *m/e* 257; 16-NAMA, *m/e* 285; 18-NAMA, *m/e* 313], GC–TEA, normal phase HPLC–TEA (different retention times for *E* and *Z* isomers), high-resolution fast atom bombardment MS (for elemental analysis), and carbon-13 nuclear magnetic resonance spectrometry (different α-, and β-carbon resonances for *E* and *Z* isomers)[3].

RESULTS AND DISCUSSION

The efficient functionalization of the hydrocarbonaceous quaternary ammonium molecules ABDAC (Fig. 1) by trichloroethoxycarbonylation[1], cyanogenation[1], or nitrosation[3] considerably extends the analytical potential of chemical derivatization methodology for trace analysis of long chain alkylated ammonium cations in complex environmental and tissue samples by using suitable group-specific detectors coupled with either GC or HPLC systems. Since a vast majority of industrial and environmental analyses involves samples with sufficiently high levels of ABDAC salts to be quantifiable directly by HPLC–UV without further structural modification, we elected to examine in greater detail the HPLC behavior of the four ABDAC homologues along with their new NAMA nitroso–derivatives (Fig. 1). In concurrent studies dealing with the two series (ABDAC and NAMA) of homologues with inherent structural dissimilarity, special attention was placed on delineating the ionic and dipolar structural characteristics of respective ABDAC and NAMA compounds in the context of retention properties under the influence of chromatographic variables. With respect to stationary phase effects, three reversed-phase columns with octadecyl-, cyanopropyl-, and phenylpropylsilica stationary phases were used. Of particular interest to us was the choice of a phenyl stationary phase in comparative studies for probing possible implication of some degrees of π–π interactions between the phenyl groups of the ABDAC solutes and of the stationary phase in the reversed-phase HPLC separation process. Such interactions would apparently be absent for the separation of NAMA compounds that are devoid of phenyl groups.

GC

We examined the GC retention behavior of nitrosamine NAMA homologues on various stationary phases (see Experimental section for column specifications). In each case, a linear correlation was found between the logarithmic relative retention and the number of carbons on the long alkyl chain. This can be expressed by the following simple relationship:

$$\ln t/t_{12} = aN + b$$

where t denotes the retention time of the homologue in question, t_{12} the retention

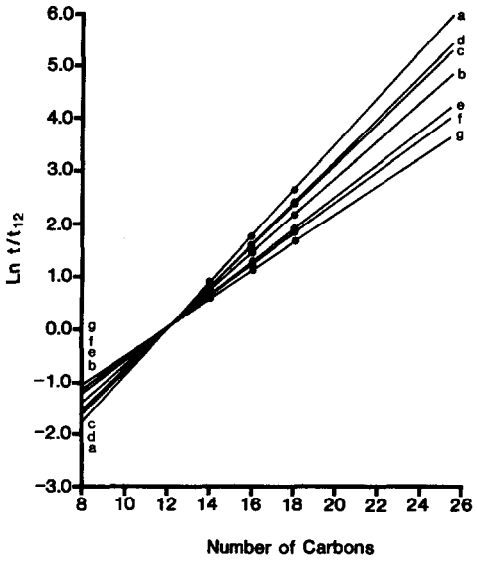

Fig. 2. Logarithmic relative retention of NAMA compounds *vs.* number of carbons on the alkyl chain. See text and Table I for GC conditions.

time of the reference homologue (12-NAMA), a the slope, N the number of carbons, and b the intercept. The plot in Fig. 2 reveals a set of straight lines each corresponding to the above equation. The slope and intercept values are listed in Table I. On the basis of these correlation data, it is possible to identify or predict unknown members of the homologue series. Discrepancies between experimental and predicted values of ln t/t_{12} for 8-NAMA were generally small, as the pertinent examples in Table I demonstrate. For practical application in trace analysis, the magnitude of the slope values (which measure the rate of the change in component retention in response to the change in carbon chain length) also provides useful information about the ex-

TABLE I

RELATION (ln $t/t_{12} = aN + b$) BETWEEN RELATIVE RETENTION, t/t_{12}, AND THE NUMBER, N, OF CARBONS ON THE ALKYL CHAIN IN NAMA HOMOLOGUES (GC)

r = Correlation coefficient obtained from regression analysis.

GC conditions*	*Slope a*	*Intercept b*	*ln t_8/t_{12}** (calculated)*	*ln t_8/t_{12} (found)*	*r*
a	0.4444	−8.1504	−1.7703	−1.7739	0.999
b	0.3613	−6.0031	−1.4011	−1.4023	0.999
c	0.3917	−6.4205	−1.5604	−1.5645	0.998
d	0.4003	−6.6201	−1.6212	−1.6208	0.998
e	0.3143	−4.4002	−1.2213	−1.2239	0.999
f	0.3009	−4.2008	−1.1601	−1.1607	0.997
g	0.2727	−3.8010	−1.0605	−1.0619	0.999

* For stationary phase details, see Experimental and Results and discussion. Column length and temperature for a–d: 100 cm, 180°C; for e and f: 100 cm, 210°C; for g: 200 cm, 220°C.

** Intercept values at $N = 8$; t_8 = retention time of 8-NAMA; t_{12} = retention time of 12-NAMA.

ponential spread of homologous components on a chromatogram obtained isothermally. For instance, the slope of the lowest line (marked as g) in Fig. 2 is 0.2727 (Table I); this is indicative of a narrowest exponential dispersion of the component peaks attainable within the shortest time scale among all the experiments (a → g) performed. Under the most favorable experimental conditions (g), simultaneous quantification of the four NAMA components at trace levels was greatly facilitated by virtue of the absence of peak broadening among the higher members of the series. When the slope of a line became steeper as did those in the upper region of the plot (Fig. 2), we found it necessary to resort to a temperature programming technique to circumvent the analytical difficulties caused by severe peak broadening of the higher members of the series.

The retention behavior of the NAMA compounds on each of three stationary phases (f), (f'), and (f") (see Experimental section for details) appeared to be identical, inasmuch as the correlation lines coincided (line f shown in Fig. 2). It should be pointed out, however, that the column length and temperature were 200 cm and 220°C for both (f') and (f") but 100 cm and 210°C for (f). In such a case, the composite influence of several chromatographic variables is believed to be operative and their individual effects become difficult to resolve. On the other hand, when comparing the result of employing one type of stationary phase with that derived from another type under identical GC conditions (*e.g.*, a *vs.* b in Table I and Fig. 2), one is led to the conclusion that stationary phase effects on the retention behavior of the NAMA components among the different column packings must be significantly dissimilar and that higher members of the series are more likely to be retained by methyl silicone packings of the OV type than by polyglycol packings of the Carbowax 20M type. The potential of the GC method for the separation of NAMA homologues was

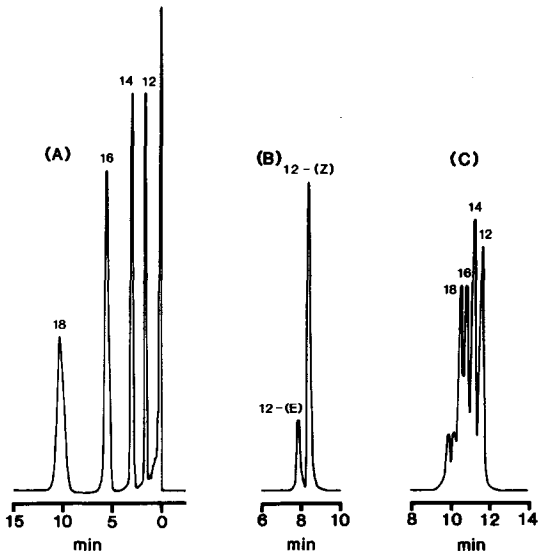

Fig. 3. Typical GC (A) and normal-phase HPLC (B and C) chromatograms showing separation of 12-, 14-, 16-, and 18-NAMA homologues. (A):GC conditions are as for (e) in Fig. 2. (B and C): HPLC conditions, hexane–[THF–methylene chloride–ISP(1:1:0.1)] (97:3).

realized in the sensitive and specific analyses of mixtures of the quaternary ammonium ABDAC homologues at ppb levels. In these analyses, a thermal energy analyzer was used for detection of the ABDAC salts as the NAMA derivatives. A typical chromatogram showing adequate separation of the four homologous NAMA components is given in Fig. 3A. The chromatographic profiles obtained independently with a flame ionization detector and a thermal energy analyzer under the same GC conditions were indistinguishable.

Normal-phase HPLC

In light of the incompatibility of the common reversed-phase HPLC solvents with operation of the TEA interface system, many normal-phase HPLC experiments were conducted in attempts to resolve the mixture of four nitroso-compounds (12-, 14-, 16-, and 18-NAMA). All were unsuccessful. The stationary phases investigated in the normal-phase mode included silica and cyanopropylsilica (see Experimental section for details). Neither stationary phase exhibited enough selectivity to achieve reasonable resolution of the nitrosamine components. Thus, elution of the nitrosamine mixture through a standard cyano-column (25 cm × 4.6 mm I.D.) with a more polar mobile phase yielded a single sharp peak ($\alpha = 1$ for all three adjacent pairs of the four components). All attempts at separation with a variety of less polar mobile phases and other cyano-columns, including two standard columns coupled in series (overall dimensions, 50 cm × 4.6 mm I.D.), led to either no separation or to an ill-defined band. Experiments with the standard silica column, which contains high-efficiency spherical microparticulate packings, were somewhat more revealing. As illustrated in Fig. 3C, the four NAMA homologues were partially resolvable on this column under the mobile phase conditions specified. Under similarly favorable conditions, the *E* and *Z* forms (Fig. 1) that arise from the configurational isomerism of a nitrosamine homologue were well separated (Fig. 3B). The two early eluting minor peaks in Fig. 3C are undoubtedly attributable to the *E* isomers of 16- and 18-NAMA homologues. These data, in concert with some of those from our earlier studies on analogous compounds[5], establish the importance of polarity effects (as a result of hindered rotation around the N–N bond in NAMA) in controlling the normal-phase separation process where adsorption is presumably the predominant mechanism of interaction between NAMA solutes and silica. Although the goals we initially set for baseline separation of the NAMA homologues were not fully met, the application of the normal-phase HPLC technique to the preparative separation of the *E*–*Z* isomers of individual NAMA homologues has been successful, and enabled the sensitive analysis of the four NAMA components as a total nitrosamine mixture in various test samples.

Reversed-phase HPLC

Representative chromatograms obtained from reversed-phase HPLC of the quaternary ammonium homologues (ABDAC) are shown in Fig. 4 and related nitroso-alkylmethylamines (NAMA) in Fig. 5. Evidently there was a fundamental difference in the retention behavior between the two series (ABDAC and NAMA) in that the elution of the former required the presence of added counter ions and the separation of its homologous constituents was dramatically affected by the combined effects of the mobile and stationary phases. Inspection of the peak patterns in Fig.

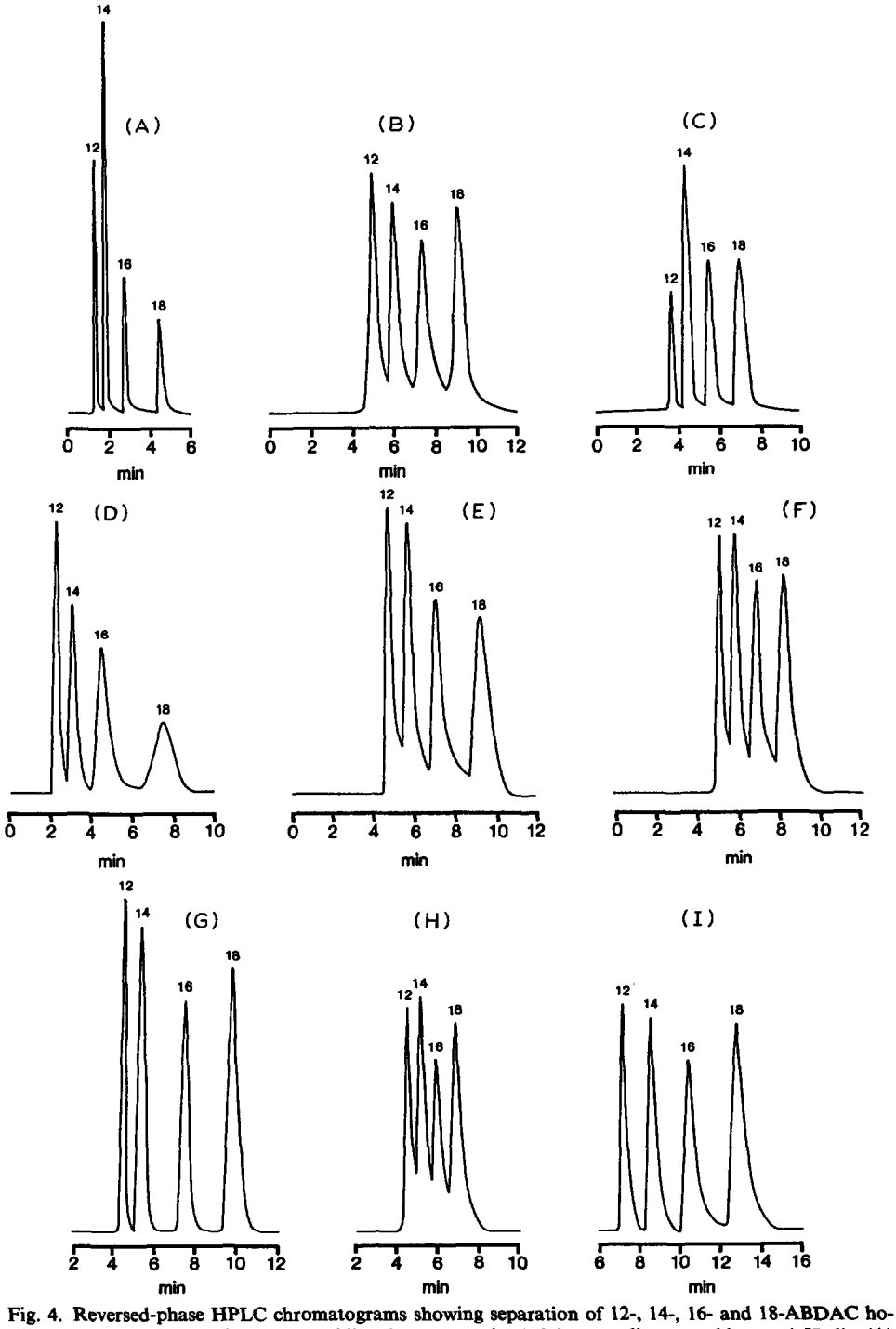

Fig. 4. Reversed-phase HPLC chromatograms showing separation of 12-, 14-, 16- and 18-ABDAC homologues. HPLC conditions: all mobile phases contained 0.1 *M* sodium perchlorate (pH 3); (A) acetonitrile–water (9:1), (B) acetonitrile–water (1:1), (C) acetonitrile–water (7:3), (D) methanol–water (9:1), (E) methanol–water (3:2), (F) methanol–water (17:3), (G)THF–water (3:2), (H) THF–water (1:1), (I) THF–water (3:2); stationary phases, (A, D, G) octadecylsilica (ODS), (B, E, H) cyanopropylsilica (CPS), (C, F, I) phenylpropylsilica (PPS).

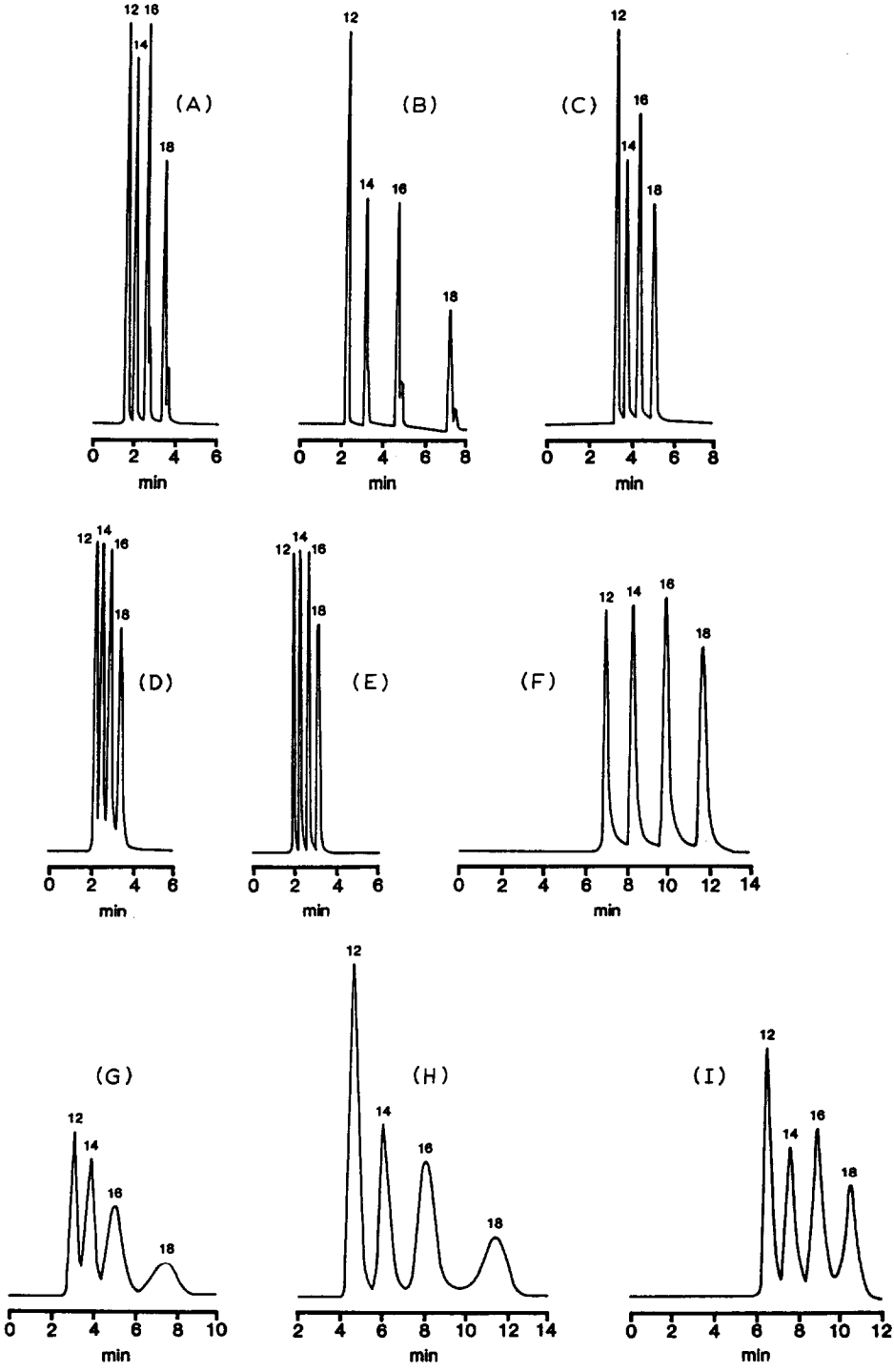

Fig. 5. Reversed-phase HPLC chromatograms showing separation of 12-, 14-, 16- and 18-NAMA homologues. HPLC conditions: mobile phases (A) acetonitrile, (B) methanol, (C) THF–water (7:3), (D) acetonitrile–water (9:1), (E) methanol–water (4:1), (F) THF–water (3:2), (G) acetonitrile–water (3:2), (H) methanol–water (9:11), (I) THF–water (1:1); stationary phases, ODS (A–C), PPS (D–F), CPS (G–I).

4 reveals some interesting features. Among the three stationary phases (octadecyl-, cyanopropyl-, and phenylpropylsilica) used in this work, the octadecylsilica packings proved to be the most satisfactory column of choice for obtaining a superior separation of the ABDAC mixture and simultaneously attaining the best degree of peak symmetry for each component separated (Fig. 4A,D,G). Except in one case where the phenypropylsilica column was used as the stationary phase and acetonitrile as the organic modifier in the mobile phase (Fig. 4C), reversed-phase HPLC of the ABDAC homologues on a moderately polar stationary phase (phenypropyl- or cyanopropylsilica) afforded fairly well resolved peaks. In each case, however, the separation was accompanied by various degrees of unavoidable peak tailing. We suggest that the peak tailing problem is probably associated with the adsorption of the quaternary cations on polar sites of the stationary phase being used. In a few instances, the adsorption process may occur in acute competition with the partition mechanism by which the ABDAC solutes are generally believed to participate preferentially in solvophobic interactions during the reversed-phase HPLC separation process.

Comparisons of the chromatograms obtained with different mobile phases (Figs. 4 and 5) indicated that the type of an organic modifier (methanol, acetonitrile, or THF) in a mobile phase can play a pivotal role in determining the retention behavior of homologous compounds under the influence of stationary phase conditions. Even so, the overall effect of both the mobile and stationary phases on peak symmetry factors involving the NAMA components was found to be a minor one (Fig. 5). This noticeable difference in peak characteristics may reflect the variation in structures between the two homologous series. In the interest of structural consideration, we believe it is important and useful to compare the extent of peak separation between an *E–Z* pair and an adjacent homologous pair in the NAMA series. Retention measurements on relevant components point to remarkable differences in the magnitude of relative retention between such pairs of disparate structural origin. Thus, the observed order of the differential retentivity is $\Delta k'$ (*E–Z*) < Δ k' [(14-NAMA)–(12-NAMA)] < $\Delta k'$ [(16-NAMA)–(14-NAMA)] < $\Delta k'$ [(18-NAMA)–(16-NAMA)]. This is in sharp contrast with that observed in normal-phase HPLC (see earlier discussion in this section). This situation also contrasts with analogous cases reported previously by Abidi[5], where retention differences between *E–Z* configurational isomers were invariably larger than those between *cis–trans* geometrical isomers as observed in both normal-phase and reversed-phase HPLC. In the two upper chromatograms in Fig. 5A and 5B, there are two sets of slightly resolved peaks, each assignable to isomers *E* (the minor peak) and *Z* (the major peak) of 16-NAMA and 18-NAMA. In most cases, it was not difficult to optimize the reversed-phase HPLC conditions to achieve excellent resolution of *E–Z* isomers. However, no separation of any pair of the configurational isomers was detected when either THF or a cyanopropylsilica column was used in conjunction with other reversed-phase systems.

Table II and Table III contain selected reversed-phase HPLC data for respective ABDAC and NAMA homologous series to show the influence of chromatographic variables on the capacity factor (k'), resolution (R), and selectivity (α) parameters. It is clear from the data that, for both series, a reduction in the percentage of an organic modifier in a mobile phase predictably resulted in improvement of component resolution (higher R values) and column selectivity (higher α values). In order

TABLE II

SELECTED REVERSED-PHASE HPLC DATA FOR ABDAC HOMOLOGUES

Resolution (R) and selectivity (α) values were determined based on adjacent components. Capacity factors (k') were mean values of three determinations.

*HPLC conditions, percentage organic modifier**	*Component*								
	12-ABDAC			*14-ABDAC*			*16-ABDAC*			*18-ABDAC*
	k'	R	α	k'	R	α	k'	R	α	k'
Octadecylsilica										
95% Methanol (0.1)	0.51	0.98	(1.63)	0.83	0.89	(1.51)	1.25	1.14	(1.66)	2.08
90% Methanol (0.1)	0.80	1.20	(1.65)	1.32	1.59	(1.92)	2.54	1.70	(1.91)	4.85
95% Acetonitrile (0.1)	0.40	1.75	(1.53)	0.61	1.60	(1.85)	1.13	1.64	(1.88)	2.12
90% Acetonitrile (0.01)	0.75	0.75	(1.49)	1.12	1.67	(2.13)	2.38	1.71	(1.94)	4.62
90% Acetonitrile (0.05)	0.82	1.23	(1.52)	1.25	2.50	(2.00)	2.50	2.92	(1.95)	4.88
90% Acetonitrile (0.25)	0.85	2.40	(1.76)	1.50	4.17	(2.04)	3.06	5.73	(1.88)	5.75
60% THF (0.1)	1.25	1.64	(1.52)	1.90	2.28	(1.39)	2.65	3.75	(1.57)	4.15
50% THF (0.1)	1.74	2.17	(1.61)	2.81	2.89	(1.63)	4.59	4.18	(1.49)	6.84
Phenylpropylsilica										
90% Methanol (0.1)	0.52	0.81	(1.17)	0.61	0.83	(1.23)	0.75	0.86	(1.24)	0.93
85% Methanol (0.1)	0.77	1.27	(1.27)	0.98	1.54	(1.34)	1.31	1.65	(1.37)	1.79
80% Acetonitrile (0.1)	1.10	0.53	(1.06)	1.17	0.89	(1.28)	1.50	0.80	(1.22)	1.83
70% Acetonitrile (0.01)	4.26	0.50	(1.31)	5.58	0.69	(1.36)	7.58	0.70	(1.37)	10.4
70% Acetonitrile (0.05)	2.67	1.33	(1.31)	3.50	1.45	(1.38)	4.83	1.41	(1.33)	6.42
70% Acetonitrile (0.1)	2.27	1.41	(1.30)	2.95	1.85	(1.39)	4.10	1.79	(1.31)	5.37
70% Acetonitrile (0.25)	2.00	1.64	(1.35)	2.69	2.07	(1.35)	3.62	2.29	(1.34)	4.85
60% THF (0.1)	1.21	1.37	(1.31)	1.59	1.45	(1.31)	2.09	1.63	(1.30)	2.71
50% THF (0.1)	2.74	2.60	(1.39)	3.43	2.86	(1.22)	4.18	3.15	(1.35)	5.64
Cyanopropylsilica										
60% Methanol (0.1)	1.56	1.18	(1.32)	2.06	1.30	(1.40)	2.89	1.40	(1.42)	4.11
50% Methanol (0.1)	5.47	1.60	(1.60)	8.76	1.83	(1.77)	15.5	2.42	(1.91)	29.6
50% Acetonitrile (0.01)	12.6	0.95	(1.29)	16.3	1.22	(1.29)	21.1	1.24	(1.24)	26.1
50% Acetonitrile (0.05)	4.51	1.13	(1.28)	5.75	1.25	(1.15)	6.59	1.30	(1.40)	9.25
50% Acetonitrile (0.20)	3.38	1.32	(1.22)	4.13	1.66	(1.27)	5.25	1.86	(1.26)	6.63
40% Acetonitrile (0.1)	8.6	2.25	(1.42)	12.2	2.68	(1.44)	17.6	3.00	(1.47)	25.8
50% THF (0.1)	2.07	0.93	(1.22)	2.53	1.07	(1.21)	3.07	1.23	(1.19)	3.67
40% THF (0.1)	4.06	2.00	(1.37)	5.56	2.13	(1.36)	7.56	2.32	(1.36)	10.3

* Values in parentheses are molar concentrations of perchlorate buffers, pH 3.

to maintain R and α values comparable to those obtained from experiments with the non-polar stationary phase, lesser amounts of an organic modifier were required in experiments with more polar stationary phases. Regardless of the stationary phase used, the reversed-phase separations of the ABDAC homologues (Table II) showed salts effects that were consistently opposite to those of the NAMA homologues (Table III). It is interesting to note that the steady increases in R values with increasing perchlorate concentrations are shown by all adjacent ABDAC components, while the corresponding α values fluctuate slightly. The addition of the perchlorate salt to mobile phases was found to cause decreases in R values of all adjacent NAMA components. No definite trend of changes in the corresponding α values can be ex-

TABLE III

SELECTED REVERSED-PHASE HPLC DATA FOR NAMA HOMOLOGUES

For R and α determinations, see Table II.

HPLC conditions, percentage organic modifier in water	*Component*								
	12-NAMA			*14-NAMA*			*16-NAMA*			*18-NAMA*
	k'	R	α	k'	R	α	k'	R	α	k'
Octadecylsilica										
100% Methanol	0.89	2.00	(1.49)	1.33	3.00	(1.50)	2.00	4.00	(1.45)	2.89
90% Methanol	3.27	8.86	(1.86)	6.09	13.0	(1.97)	12.0	14.7	(1.95)	23.4
100% Acetonitrile	1.32	4.50	(1.67)	2.21	6.00	(1.69)	3.75	8.33	(1.66)	6.20
90% Acetonitrile	3.06	11.6	(1.93)	5.92	14.3	(1.96)	11.6	19.0	(1.98)	23.00
90% Acetonitrile*	3.75	8.29	(1.97)	7.38	9.33	(1.95)	14.4	12.3	(1.97)	28.3
80% THF	0.67	0.80	(1.12)	0.75	1.03	(1.17)	0.88	1.33	(1.18)	1.04
70% THF	1.19	1.45	(1.21)	1.44	2.04	(1.26)	1.81	2.33	(1.24)	2.25
Phenylpropylsilica										
90% Methanol	0.47	0.85	(1.43)	0.67	1.12	(1.45)	0.97	1.38	(1.37)	1.33
80% Methanol	2.05	3.67	(1.77)	3.62	4.55	(1.66)	6.01	5.72	(1.66)	9.95
80% Acetonitrile	0.90	1.50	(1.36)	1.22	1.75	(1.27)	1.55	2.44	(1.36)	2.11
70% Acetonitrile	1.71	2.67	(1.47)	2.51	2.43	(1.33)	3.35	3.18	(1.40)	4.70
70% Acetonitrile*	2.25	2.11	(1.37)	3.08	2.67	(1.38)	4.25	2.92	(1.37)	5.83
70% THF	0.57	1.00	(1.18)	0.67	0.80	(1.13)	0.76	0.62	(1.13)	0.86
60% THF	2.32	2.36	(1.25)	2.91	2.67	(1.25)	3.64	2.77	(1.22)	4.45
Cyanopropylsilica										
60% Methanol	2.20	0.89	(1.37)	3.08	0.64	(1.35)	4.16	1.02	(1.57)	6.53
50% Methanol	3.63	1.54	(1.78)	6.47	1.45	(2.05)	13.3	2.00	(1.91)	25.3
50% Acetonitrile	2.38	1.20	(1.31)	3.12	1.43	(1.28)	4.00	1.08	(1.31)	5.25
50% Acetonitrile*	1.76	1.19	(1.27)	2.24	1.40	(1.27)	2.85	1.21	(1.34)	3.83
45% Acetonitrile	3.35	1.51	(1.37)	4.60	1.68	(1.41)	6.47	1.94	(1.44)	9.29
50% THF	3.33	1.12	(1.22)	4.07	1.34	(1.21)	4.93	1.61	(1.22)	6.00
40% THF	8.27	1.77	(1.39)	11.5	2.15	(1.42)	16.3	2.15	(1.38)	22.5

* Containing perchlorate buffer, 0.1 M, pH 3.

tracted from the data in Table III. In view of the lack of striking differences in the chromatographic characteristics demonstrated by ABDAC components in comparison with those by the NAMA counterparts, it seems logical to preclude implications of π–π interactions between the phenyl groups of the phenylpropylsilica stationary phase and of the ABDAC compounds in the reversed-phase separation process.

The k'–% water profiles for reversed-phase HPLC of ABDAC compounds (Fig. 6) are surprisingly similar to those of NAMA compounds (Fig. 7). As clearly envisaged from the graphs in Figs. 6 and 7, the retention behavior of the compounds in either series on octadecylsilica depends, to an important degree, on the nature of the organic modifier used in the mobile phase. Substitution of the non-polar stationary phase with a moderately polar one (phenylpropyl- or cyanopropylsilica) leads to comparatively minor mobile phase effects. More explicitly, the retention curves on each of the graphs for the latter cases are more compact and are confined to a smaller area. Further, results with the octadecylsilica stationary phase indicate that the op-

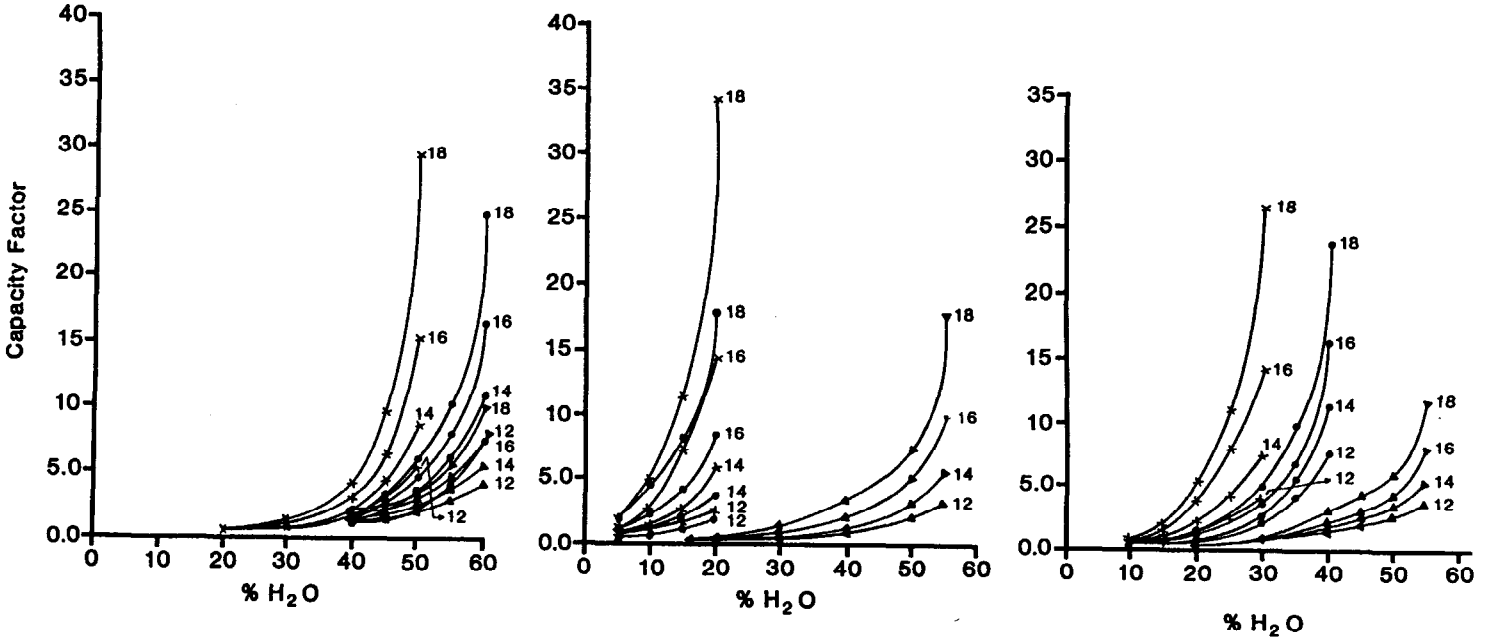

Fig. 6. Effects of % organic modifiers on k' values of ABDAC homologues studied under various stationary phase conditions. All HPLC mobile phases contained 0.1 *M* sodium perchlorate (pH 3). Symbols for organic modifiers: methanol (×), acetonitrile (●), THF (▲). Stationary phases, CPS (left), ODS (middle), PPS (right).

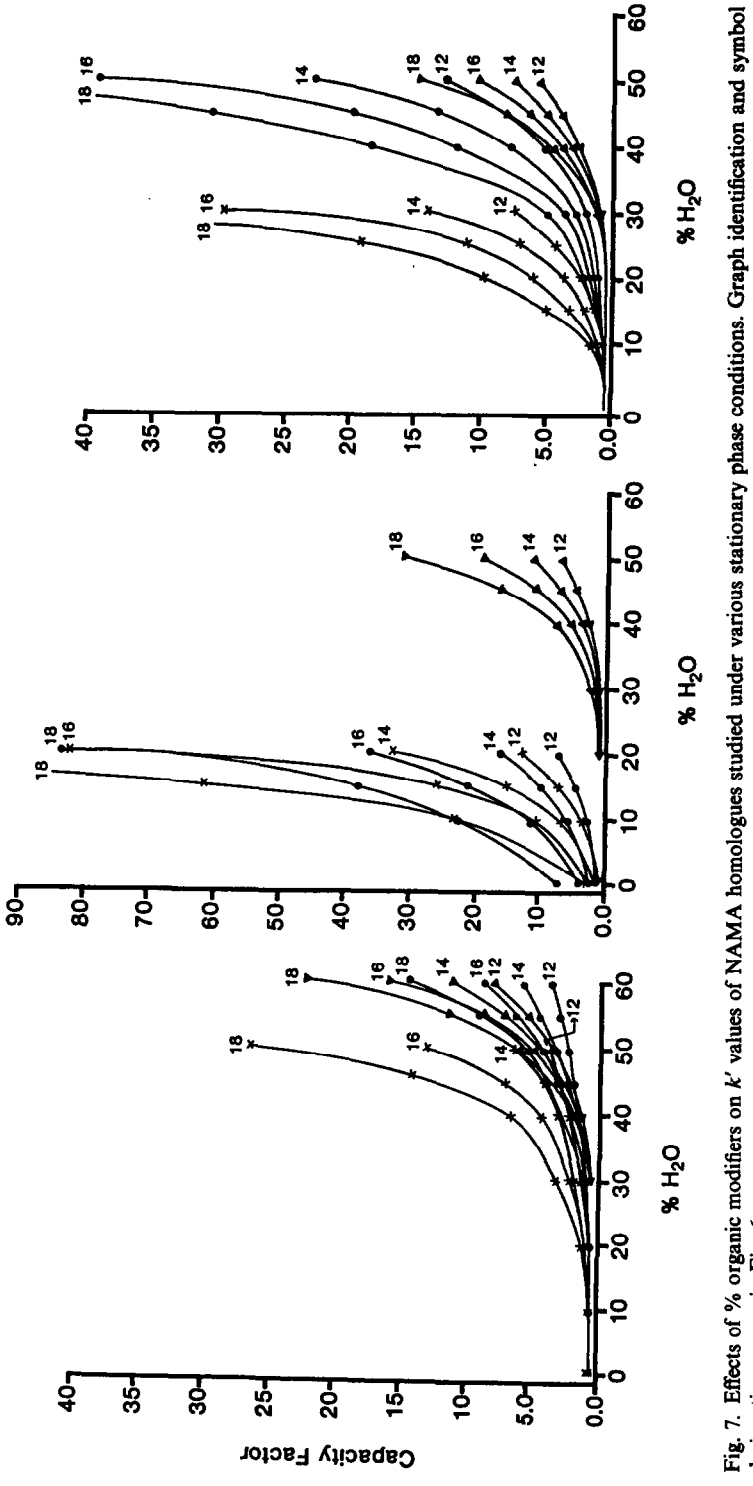

Fig. 7. Effects of % organic modifiers on k' values of NAMA homologues studied under various stationary phase conditions. Graph identification and symbol designations are as in Fig. 6.

timal mobile phase solvent compositions shown along % water axes (Figs. 6 and 7) vary with the type (methanol, acetonitrile, or THF) of mobile phases in a unique fashion reflective of the order of solvent strength parameters for reversed-phase HPLC, methanol (3.0) < acetonitrile (3.1) < THF (4.4)[6,7]. From a practical point of view, the retention data in Figs. 6 and 7 provide useful information on the optimal HPLC conditions under which routine analyses of both ABDAC and NAMA homologues with k' values in the normal range can be conveniently performed.

An insight as to the effect of changing the counter-ion concentrations on the retention behavior was secured from a brief study using the perchlorate salt buffered at pH 3. Some parallelism in stationary phase effects exists between the reversed-phase HPLC results of the two series of title compounds. Fig. 8 illustrates the definite stationary effect observed following incorporation of the perchlorate ion in the mobile phase during the separation of the ABDAC mixture. Such a stationary phase effect in the case of the NAMA compounds appeared to occur to a much lesser extent (Fig. 9). This analogous retention behavior is suggestive of a significant contribution by the dipolar form of the NAMA resonance hybrid[8]. In all cases, concentration effects were found to be most significant at low concentrations (below 0.1 *M*). Over the entire higher concentration range (0.1–0.5 *M*), the capacity factors were essentially invariant with the changes in salt concentrations. Examination of the retention curves (Figs. 8 and 9) obtained from experiments in which three reversed-phase columns of different polarity were used shows that the initial upward and downward

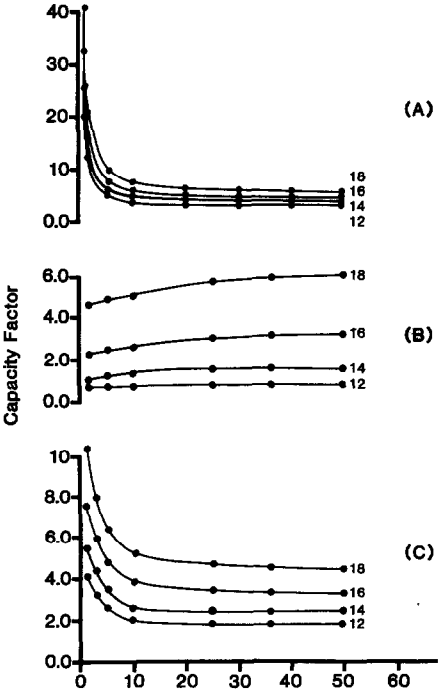

Fig. 8. Effects of perchlorate concentrations on k' values of ABDAC components studied under various stationary phase conditions. HPLC conditions: mobile phases (pH 3), acetonitrile–water, 1:1 (A), 9:1 (B), 1:1 (C); stationary phases, CPS (A), ODS (B), PPS (C).

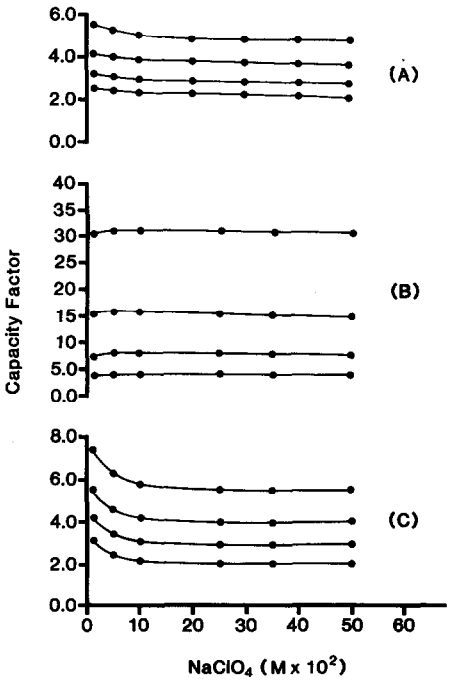

Fig. 9. Effects of perchlorate concentrations on k' values of NAMA components studied under various stationary phase conditions. HPLC conditions and graph identification are as in Fig. 8.

trends of k' values associated with small increases in the perchlorate concentration demonstrate different stationary phase effects operating during the HPLC processes. We presume that this manifestation of apparently opposite stationary phase effects reflects different separation mechanisms for reversed-phase HPLC of the ABDAC components under various stationary phase conditions. In the absence of additional data, however, we are presently unable to explain the observed chromatographic phenomena in terms of a mechanistic rationale based on ion-pair, ion-exchange, and ion-interaction considerations[9].

In a related study, we examined the counter-ion effect on the capacity factor (k') and resolution (R) parameters of the cationic ABDAC components using a series of homologous alkyl sulfonic acid salts as counter-ions in the mobile phases (Table IV). The results with perchlorate and phosphate salts are also included for comparison. Evidently, the addition of a more hydrocarbonaceous counter-ion to the mobile phase resulted in moderately higher k' values of the quaternary ammonium analytes. This conforms with conventional generalities of ion-pair reversed-phase HPLC. In light of the straight-chain form of the alkyl chain in ABDAC, it did not seem unusual to find that ABDAC components were more retained by the stationary phase with octane sulfonate (straight-chain structure) as the counter-ion than with dimethylcyclohexane sulfonate (cyclic structure). Both counter-ions have the same number of carbons in the molecule. Of the eight counter-ions studied, the system in which the perchlorate was used as counter-ion in the mobile phase appeared to be most advantageous, as judged from the retention and resolution data. On the other hand,

TABLE IV

COUNTER-ION EFFECT ON THE CAPACITY FACTOR (k') AND RESOLUTION (R) PARAMETERS OF ABDAC COMPOUNDS*

Each HPLC mobile phase contained 0.1 M sodium salt of the counter ion of interest (pH 3).

Component	*Counter-ion**							
	DPH	*MS*	*PS*	*BS*	*HS*	*OS*	*DMCHS*	*PC*
Octadecylsilica (acetonitrile–water, 9:1)								
12-ABDAC								
k'	4.02	0.81	1.00	1.11	1.33	1.55	1.13	0.93
R	1.50	2.25	1.73	1.78	1.53	1.57	1.87	2.40
14-ABDAC								
k'	7.07	1.52	2.00	2.13	2.33	2.67	2.01	1.74
R	1.60	1.86	1.76	1.62	2.19	2.11	2.29	2.50
16-ABDAC								
k'	13.0	3.03	3.54	3.78	4.33	5.00	3.80	3.41
R	1.44	2.00	1.69	1.80	2.00	2.05	2.56	2.72
18-ABDAC								
k'	24.5	5.81	6.31	6.55	7.55	8.78	6.90	6.20
Phenylpropylsilica (acetonitrile–water, 7:3)								
12-ABDAC								
k'	2.88	1.88	2.00	2.06	2.24	2.63	2.14	2.29
R	1.29	1.42	1.33	1.56	1.47	1.65	1.60	1.81
14-ABDAC								
k'	4.06	2.53	2.65	2.71	3.06	3.50	2.86	3.00
R	1.35	1.54	1.30	1.44	1.60	1.65	1.70	1.89
16-ABDAC								
k'	5.65	3.47	3.65	3.71	4.18	4.50	3.86	4.04
R	1.16	1.38	1.33	1.37	1.50	1.56	1.48	1.95
18-ABDAC								
k'	7.82	4.65	5.00	5.27	5.71	6.31	5.14	5.47

* Abbreviations: DPH = dihydrogen phosphate, MS = methane sulfonate, PS = propane sulfonate, BS = butane sulfonate, HS = hexane sulfonate, OS = octane sulfonate, DMCHS = dimethylcyclohexane sulfonate, PC = perchlorate.

related data from Table IV on the use of a phosphate salt as the sole counter-ion source in the mobile phase disclose the least satisfactory chromatographic characteristics of the ABDAC components.

Since the ABDAC compounds are fully charged molecules, it was plausible to investigate the pH-dependence of k' values under different stationary phase conditions. Capacity factors of all components were determined over a pH range of 2–6 in phosphate buffers. As shown in Fig. 10, the pH–k' plots exhibit three different sets of curves, the curvature depending upon the type of stationary phase used in the reversed-phase separation. This is a clear indication of some interplay between stationary phase effect and pH effect on component retentivity. Nevertheless, there was a general tendency, in all instances, for the capacity factor to be higher in response to an increase in eluent pH, especially in the higher pH range (4–6). This trend in pH effect, which is seemingly characteristic of cationic amine compounds, reminded us of our previously published results on quinoidal imminium compounds[10]. We also

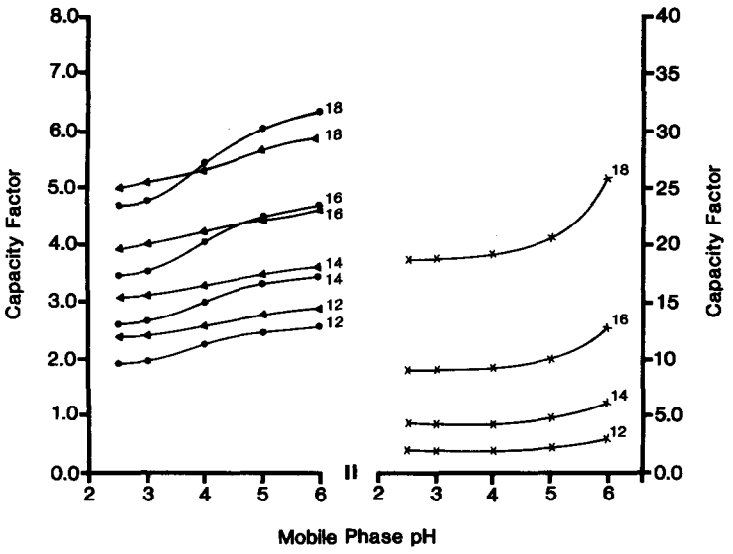

Fig. 10. pH dependence of the capacity factor, k', of ABDAC components studied under various stationary phase conditions. HPLC conditions: all mobile phases contained 0.1 M sodium perchlorate buffers. Mobile phases, acetonitrile–water, 4:1 (×), 1:1 (▲), 7:3 (●); stationary phases, ODS (×), CPS (▲), PPS (●).

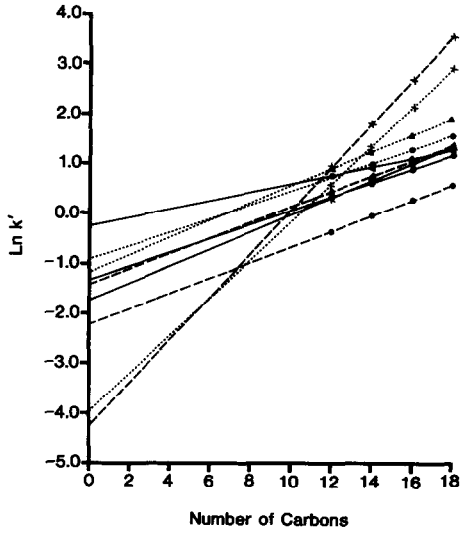

Fig. 11. Linear correlation of logarithmic k' with the alkyl chain length in ABDAC homologues. HPLC conditions: mobile phases, acetonitrile–water (·····), methanol–water (– – –), THF–water (———); symbols for stationary phases are as in Fig. 10. See Table V for other details.

note from Fig. 10 that the mobile phase pH seems to exert a greater influence on the k' values of the higher members of the homologous series because the upward swing of the retention curve for 16-ABDAC or 18-ABDAC is clearly more prominent.

Results from correlation studies on reversed-phase HPLC of the title compounds are summarized in Figs. 11 and 12 and Table V. For each homologue in both the ABDAC (Fig. 11) and the NAMA (Fig. 12) series, the linear correlation between the logarithmic capacity factor (k') and the number (N) of carbons on the alkyl chain was excellent. The relation between the two variables can best be represented by the following equation:

$$\ln k' = cN + d$$

where c is the slope and d is the intercept. This linear relationship supports the general concept that the total area of the straight-chain alkyl group is accessible for the solvophobic interactions in reversed-phase HPLC[11,12]. Table V incorporates all the slope and intercept values of the 18 straight lines (Figs. 11 and 12) obtained from various experiments under the optimized conditions specified. In analogy with the GC results described earlier, it is highly recommendable to select suitable HPLC conditions that would give acceptable correlation lines with lower slope values for trace analysis of homologous mixtures. The minimization of peak broadening and analysis with enhanced sensitivity for the higher members of the homologous mixture can then be realized. The correlation data provided in Table V can be used to predict k' values of other members in the homologous series as demonstrated in the examples for the octyl-compounds, 8-ABDAC and 8-NAMA. As noted, the logarithmic k' values calculated from the above equation are in good agreement with those found experimentally.

In conclusion, the GC separation technique provides a unique means for sen-

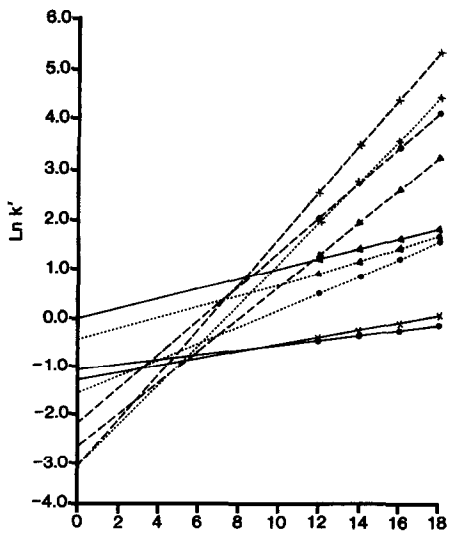

Fig. 12. Linear correlation of logarithmic k' with the alkyl chain length in NAMA homologues. See Figs. 10 and 11 and Table V for line identification and HPLC conditions.

TABLE V

RELATION ($\ln k' = cN + d$) BETWEEN THE CAPACITY FACTOR, k', AND THE NUMBER, N, OF CARBONS ON THE ALKYL CHAIN IN ABDAC AND NAMA HOMOLOGUES (HPLC)

All mobile phases for ABDAC compounds contained 0.1 *M* perchlorate buffer at pH 3. r = Correlation coefficient obtained from regression analysis.

HPLC conditions, percentage organic modifier in water	*Slope c*	*Intercept d*	*ln k'_8* (calculated)*	*ln k'_8 (found)*	*r*
Octadecylsilica			*ABDAC*		
80% Methanol	0.4333	−4.2513	−0.7963	−0.7974	0.999
80% Acetonitrile	0.3833	−3.9504	−0.9799	−0.9801	0.998
60% THF	0.1750	−1.2006	−0.3784	−0.3795	0.997
Phenylpropylsilica					
85% Methanol	0.1583	−0.9521	−0.9982	−0.9978	0.999
70% Acetonitrile	0.1502	−0.2514	0.1935	0.1929	0.999
60% THF	0.1503	−1.4007	−0.2276	−0.2301	0.998
Cyanopropylsilica					
60% Methanol	0.1583	−1.3511	−0.1540	−0.1533	0.999
50% Acetonitrile	0.1667	−1.7509	0.1978	0.1983	0.999
50% THF	0.0833	−2.2025	0.4329	0.4314	0.999
Octadecylsilica			*NAMA*		
80% Methanol	0.4667	−3.0011	0.6799	0.6805	0.999
80% Acetonitrile	0.4133	−3.0304	0.2654	0.2649	0.997
80% THF	0.0727	−1.2409	−0.7107	−0.7095	0.999
Phenylpropylsilica					
70% Methanol	0.3429	−2.6025	0.6658	0.6653	0.999
70% Acetonitrile	0.1667	−1.2813	−0.1987	−0.2009	0.998
70% THF	0.0485	−1.0201	−0.6940	−0.7010	0.999
Cyanopropylsilica					
50% Methanol	0.3294	−2.6021	−0.0395	−0.0379	0.999
50% Acetonitrile	0.1167	−0.2216	0.4753	0.4738	0.998
50% THF	0.0667	−0.0237	0.8017	0.8013	0.999

* Intercept values at $N = 8$; k'_8 = capacity factor of 8-NAMA or 8-ABDAC.

sitive TEA detection of ABDAC homologues via their NAMA derivatives. Although results from normal-phase HPLC demonstrated limited applicability of this technique in the component resolution of NAMA homologues, the potential usefulness of the normal-phase separation method for the separation and isolation of the configurational isomers should be recognized. The present reversed-phase HPLC work illustrated that significant mobile phase and stationary phase effects on the retention behavior of both the ABDAC and NAMA compounds were operative in all cases studied. The technique of using mobile phases of higher water content, as in reversed-phase HPLC on either phenylpropyl- or cyanopropylsilica, can be applied to the specific analysis of NAMA compounds by HPLC electrochemical detection.

REFERENCES

1 S. L. Abidi, *J. Chromatogr.*, 200 (1980) 216.
2 F. W. Crow and S. L. Abidi, *Paper presented at the 32nd Annual Meeting of the American Society of Mass Spectrometry, San Antonio, TX, 1980.*
3 S. L. Abidi, *Paper presented at the 189th National Meeting of the American Chemical Society, Miami, FL, 1985.*
4 S. L. Abidi, *Anal. Chem.*, 54 (1982) 510.
5 S. L. Abidi, *J. Chromatogr.*, 288 (1984) 277.
6 L. R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
7 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
8 D. R. Battiste, L. P. Davis and R. V. Nauman, *J. Amer. Chem. Soc.*, 97 (1975) 5071.
9 S. N. Deming and R. C. Kong, *J. Chromatogr.*, 217 (1981) 421, and references cited therein.
10 S. L. Abidi, *J. Chromatogr.*, 255 (1983) 101.
11 H. Colin and G. Guiochon, *J. Chromatogr.*, 141 (1977) 289.
12 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21.