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LIQUID CHROMATOGRAPHY OF HYDROCARBONACEOUS QUATERNARY AMINES ON CYCLODEXTRIN-BONDED SILICA

S. L. ABIDI
U.S. Fish and Wildlife Service, National Fishery Research Laboratory, P.O. Box 818, La Crosse, WI 54602-0818 (U.S.A.)
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SUMMARY

Mixtures of *n*-alkylbenzyldimethylammonium chloride (ABDAC) were resolved into homologous components by high-performance liquid chromatography (HPLC) with a cyclodextrin-bonded silica stationary phase. With a few exceptions, results from this study are similar to those obtained from traditional reversed-phase HPLC. It was found that the presence of electrolytes in aqueous mobile phases is not a critical factor in determining the success of HPLC separation. Under normal HPLC conditions, a mobile phase consisting of either methanol–water (50:50) or acetonitrile–water (30:70) was employed for obtaining adequate resolution of the quaternary ammonium mixtures. Although the percent organic modifier–water profiles were similar to those in previous studies with these compounds, resolution (R) and selectivity (α) parameters were found to be quite susceptible to changes in the mobile phase solvent composition. The retention behavior of the cationic analytes in the homologous series is consistent with the hydrophobic-interaction concept proposed for the retention mechanism via dominant inclusion complex formation. Several electolytes were chosen for a study of the counter ion effect on the chromatographic characteristics of ABDAC components. Among the electrolytes examined, the perchlorate ion was found most likely to act as an ion-pairing counter ion for ammonium cations in the HPLC system studied. A correlation study established linear relationships between the chain length of ABDAC and the logarithmic capacity factor (k'). The analytical utility of the HPLC method was demonstrated by the analysis of various unknown mixtures.

INTRODUCTION

Typical representatives of hydrocarbonaceous quaternary amines are the alkylbenzyldimethylammonium chlorides (ABDAC), in which the alkyl group is an unbranched hydrocarbon chain with the number of carbons ranging from 8 to 18. These compounds are of continuing interest to researchers in chemical and pharmaceutical industries because of their well-recognized algicidal, bactericidal, and fungicidal activities. Mixtures of quaternary ammonium compounds having various

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compositions of the active component amines are extensively used in commercial products. The four-component mixtures of ABDAC in which the alkyl substituents at the quaternary ammonium nitrogen consist of dodecyl, tetradecyl, hexadecyl, and octadecyl homologous groups are of particular interest to us. Commercially formulated materials containing these four ammonium salts have been used as therapeutants in fish culture for the treatment of bacterial gill disease.

Owing to their high lability to undergo thermal decomposition under normal gas chromatographic (GC) conditions, quaternary ammonium compounds are generally not quantifiable by GC. However, when the tetravalent nitrogen of a quaternary amine is chemically modified to the trivalent nitrogen through chemical derivatization, the parent compound can be measured by GC by using the derivatized species as an anlyte. Three chemical derivatization methods for the indirect analysis of a mixture of ABDAC by GC have been described[1,2]. In these indirect techniques, the ammonium salts were quantitatively converted to cyanamide-[1], trichloroethyl carbamate-[1], or nitroso-[2] derivatives before analysis by GC–thermionic detection, GC–electron-capture detection, or thermal energy analysis[3]. It has also been shown that high-molecular-weight quaternary ammonium compounds including ABDAC homologues, can be directly determined by high-performance liquid chromatography (HPLC)[2,4–8]. Numerous HPLC systems have been studied with respect to mobile phase and stationary phase effects on the separation of ABDAC components[2].

Our prior investigations on direct HPLC analysis of quaternary ammonium compounds indicated that, regardless of the stationary phases used, the presence of either organic or inorganic electrolytes (or counter ions) in the reversed-phase mobile phase was critical to the achievement of adequate component separation and detection sensitivity. Otherwise, as observed in experiments without added electrolytes, hydrocarbonaceous quaternary amines tended to be totally absorbed on the stationary phase employed. Prompted by these observations and a need to minimize the strong affinity of the quaternary ammonium solutes for the conventional stationary phases[2,4] in direct HPLC analysis, a study of the elution behavior of the four ABDAC salts on a cyclodextrin-bonded stationary phase was undertaken. We report here the development of an alternative HPLC procedure that can be used for direct measurement of title quaternary amines without the use of electrolytes in the mobile phase. Effects of selected chromatographic variables on the separation of ABDAC homologues are also described.

EXPERIMENTAL

Chemicals and reagents

The five ABDAC salts used in this study include decyl-(10-ABDAC), dodecyl-(12-ABDAC), tetradecyl-(14-ABDAC), hexadecyl-(16-ABDAC), and octadecylbenzyldimethylammonium chlorides (18-ABDAC). They were prepared by procedures[1] developed previously in our laboratory. The corresponding nitroso-alkylmethylamines (NAMA) were prepared by following published procedures[2,9]. Solvents for chromatography, acquired from J. T. Baker (Phillipsburg, NJ, U.S.A.), were reagent grade. Sodium alkyl sulfonates (Aldrich, Milwaukee, WI, U.S.A.) were high-purity materials. As sodium methane sulfonate was not commercially available, it was prepared according to a published procedure[2]. Silver nitrate and sodium per-

chlorate, obtained from Alpha Products (Danvers, MA, U.S.A.), were pure materials suitable for use in HPLC mobile phases. Other chemicals, including buffer salts (J. T. Baker), were analytical reagent-grade and were used without further purification.

HPLC

HPLC was performed on a Varian Model LC-5000 liquid chromatograph using a stainless-steel column (25 cm × 4.6 mm I.D.) packed with β-cyclodextrin-bonded silica[10] (Advanced Separations Technology, Whippany, NJ, U.S.A.). The instrument was equipped with an injection port consisting of a Valco CV-6-UHPa-N60 injection valve and a 10-μl loop (Valco Instrument, Houston, TX, U.S.A.). For monitoring column effluents, a variable-wavelength UV–VIS detector (Varian Varichrom) was set at 215 nm, the λ_{max} of the quaternary ammonium analyte of interest. In a typical analysis, each sample, dissolved in the same solvent as the organic modifier (methanol or acetonitrile) used in the mobile phase, was filtered through a fluoropolymer membrane and a 10-μl aliquot (30–50 μg/ml) was injected into the column. Detector responses were recorded on a Varian Model 9176 strip chart recorder. The void volume of the column was 2.1 ml, as determined by injecting a solution of sodium iodide in water, and the peak for the unretained solute was marked as the dead time. In all experiments, the column temperature was maintained at ambient temperature.

Acetonitrile and methanol were used throughout this work as the organic modifiers in mobile phases of the aqueous binary systems. When a mobile phase required acidic phosphate buffers and counter ion electrolytes, a mobile phase of water–acetonitrile was generally employed for solubility reasons. Separation in this system was optimized by using a mobile phase composed of water–acetonitrile (70:30). The counter ion concentration of the mobile phase was 0.1 *M* and the pH was adjusted to 3 by the addition of phosphoric acid. All pH values were measured in aqueous solutions. Unless otherwise specified, the flow-rate was 2.5 ml/min.

Preparative HPLC

To further purify the ABDAC salts obtained from recrystallization of the crude synthesized products[1] and to verify the structures of individual components eluting from the analytical column, preparative HPLC was carried out with samples (5–10 mg) of the quaternary ammonium mixtures whose components were either equally distributed or partly enriched. A mobile phase comprising water–methanol (50:50) was pumped through the preparative column (25 cm × 10 mm I.D.) (Advanced Separations Technology) at a flow-rate of 0.5 ml/min. Samples of fixed-volume were chromatographed into the column by means of a 100-μl injection loop housed in the Valco injection port. Fractions (0.5 ml) collected with a Buchler Linear Automatic Fraction collector were monitored with a Varian refractive index detector. Aliquots (20 μl) were withdrawn from the fractions that supposedly contained a single component and analyzed by analytical HPLC with UV detection. Fractions containing pure individual ABDAC components were then combined. Upon removal of solvent (methanol and water) by evaporation and exhaustive drying (in a drying pistol) under reduced pressure, a pure homologue of the quaternary amines was obtained (99.5–100% purity). Direct probe chemical ionization mass spectra of samples of the materials isolated from HPLC exhibited individually base peaks at *m/e* 305, *m/e* 333,

m/e 361, and *m/e* 389 attributable to the quaternary ammonium cations [$(M^+ + 1) - Cl$] of 12-, 14-, 16-, and 18-ABDAC. Mass spectral fragmentation patterns of homologous samples were superimposable with those of authentic compounds.

Analysis of unknown mixtures

For analytical application, the pure quaternary ammonium compounds prepared in the manner described above were used as standards in the construction of calibration curves. Solutions of at least six different concentrations of an ABDAC standard in methanol were prepared and three replicate determinations were made on each standard solution for measuring average peak areas. Plotting concentrations against peak areas produced four straight lines with different slopes corresponding to the four ABDAC components of a mixture. On the basis of these linear plots, the amount of each component in a test mixture was determined.

RESULTS AND DISCUSSION

The major advantage of using a cyclodextrin column in the HPLC separation of hydrocarbonaceous quaternary amines is the operational simplicity with which optimization of mobile phase conditions can be achieved. Unlike other reversed-phase systems where the success of simultaneous elution and separation of ionic solutes demands the addition of both counter ions and buffer salts to the mobile phase solvent used, HPLC of ABDAC salts on a cyclodextrin stationary phase yielded adequate separation of the homologous components when mobile phases of either methanol–water (50:50) or acetonitrile–water (30:70) were used (Fig. 1). The idea of

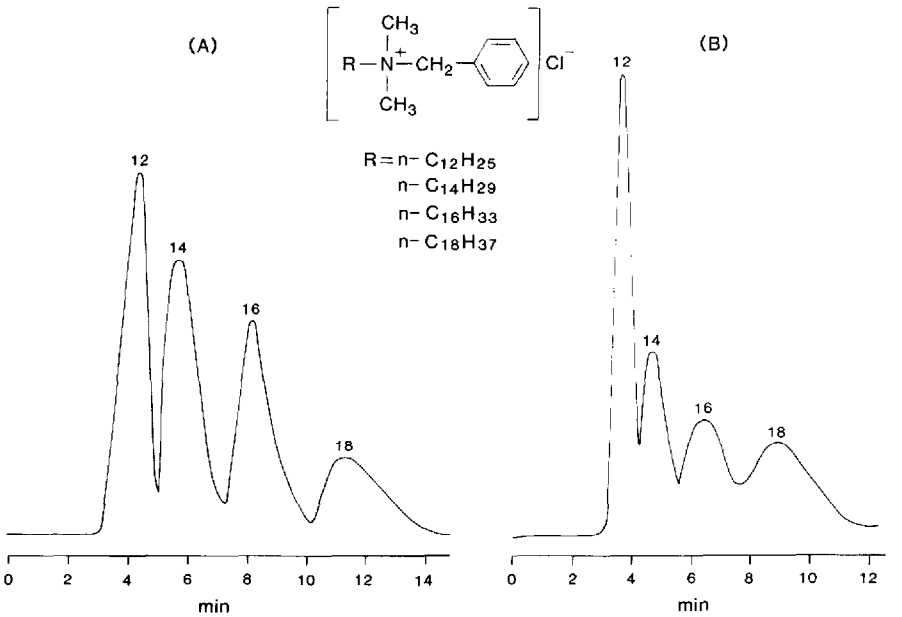

Fig. 1. Chromatographic separation of 12-, 14-, 16-, and 18-ABDAC homologues on cyclodextrin-bonded silica. Mobile phases: (A) methanol–water (50:50), (B) acetonitrile–water (30:70).

selecting hydrocarbonaceous quaternary amines to serve as complexing agents toward cyclodextrin appeared to constitute an attractive starting point for this study. In spite of a number of publications that describe the separation of enantiomers[11], diastereomers[12], and geometrical and other structural isomers[10,13] using cyclodextrin-bonded phases, there is no precedent in the literature on HPLC work with these stationary phases for the separation of homologues of any structural type. According to principles of cyclodextrin chemistry[14–16], under aqueous mobile phase conditions, solute retention is generally considered to be primarily governed by inclusion complex formation. The cyclodextrin phase employed in this work was composed of seven D(+)-glucopyranose units cyclized to form a hollow truncated cone via β-1,4 bond linkage. Hydrophobic interactions between solutes and the hydrophobic cavity of cyclodextrin are predominantly accountable for the separation mechanism by which the formation strength of inclusion complexes can be differentiated during the chromatographic process. Further, it is commonly understood that the formation constants for polar and ionic compounds including inorganic ions are usually smaller than those for non-polar and uncharged compounds. Consequently, the latter uncharged compounds are, in principle, more strongly retained by cyclodextrin than those under the former category. The situation is apparently different with the hydrocarbonaceous quaternary amine system and appears to be complicated by the concurrent presence of both polar cationic ammonium moieties and non-polar long chain alkyl groups, as in ABDAC. In accordance with the above generalization, when aqueous mobile phases of relatively high water content are used, as indicated in Fig. 1, a lessening of the formation strength in the inclusion complex of the lower member of ABDAC homologues is evidenced by a decrease in its retention time ($t_{12} < t_{14} < t_{16} < t_{18}$). In these cases where mobile phases contain either methanol–water (50:50) or acetonitrile-water (30:70), it seems reasonable to assume that the hydrophobicity of the homologous alkyl groups of interest plays an overriding role in determining resolution outcomes of the chromatographic process. Analogous effects derived from the quaternary ammonium portions around the positive charges may be insignificant.

Fig. 2 shows the influence of the mobile phase solvent composition on the capacity factor (k') of ABDAC components. The k' *vs.* % organic modifier profile bears close resemblance to that illustrated in our most recent article[2] pertaining to HPLC of ABDAC with traditional hydrocarbonaceous reversed-phase packings. Nevertheless, there are fundamental differences in the retention mechanism of the two HPLC methods. It should be reiterated that conventional reversed-phase HPLC separation of ABDAC, wherein a partition mechanism is presumably to prevail for solute retention, cannot be accomplished if mobile phases include no constituents other than solvents. In Fig. 2, we note the gradual decline in k' values with an increase in the % organic modifier. This trend is illustrative of increasing probabilities for preferential complexation of methanol (or acetonitrile) with the bonded phase. Retention data from the two solvent systems also demonstrate that weaker inclusion complexes are formed by methanol than by acetonitrile, as k' values in acetonitrile are smaller than those in methanol when measured under the same conditions. Table I presents retention (k'), resolution (R), and selectivity (α) data extracted from Fig. 2. A comparison of the R values obtained with the two types of mobile phases (acetonitrile–water *vs.* methanol–water) correlates with expectations based on pre-

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF ABDAC HOMOLOGUES

Mobile phases contained no electrolytes. R and α represent peak resolution and column selectivity for adjacent components, respectively; capacity factors (k') were mean values of three determinations.

Mobile phase (% organic modifier in water)	*Component*									
	12-ABDAC			*14-ABDAC*			*16-ABDAC*			*18-ABDAC*
	k'	R	α	k'	R	α	k'	R	α	k'
Acetonitrile										
30	2.53	0.79	(1.45)	3.69	1.07	(1.46)	5.40	1.01	(1.44)	7.77
35	1.85	0.69	(1.40)	2.60	0.97	(1.41)	3.67	0.91	(1.41)	5.19
40	1.30	0.54	(1.37)	1.79	0.80	(1.36)	2.44	0.77	(1.35)	3.30
45	0.89	0.50	(1.30)	1.16	0.73	(1.31)	1.52	0.61	(1.30)	1.98
50	0.62	0.25	(1.13)	0.70	0.28	(1.14)	0.81	0.30	(1.13)	0.88
Methanol										
50	3.32	0.81	(1.49)	4.95	1.31	(1.49)	7.39	1.15	(1.50)	11.05
55	2.56	0.79	(1.33)	3.40	1.20	(1.35)	4.60	1.10	(1.35)	6.20
60	1.86	0.71	(1.28)	2.38	1.03	(1.29)	3.12	1.00	(1.29)	4.05
65	1.53	0.56	(1.16)	1.78	0.83	(1.17)	2.08	0.77	(1.18)	2.46
70	1.32	0.00	(1.00)	1.32	0.20	(1.08)	1.40	0.00	(1.00)	1.40

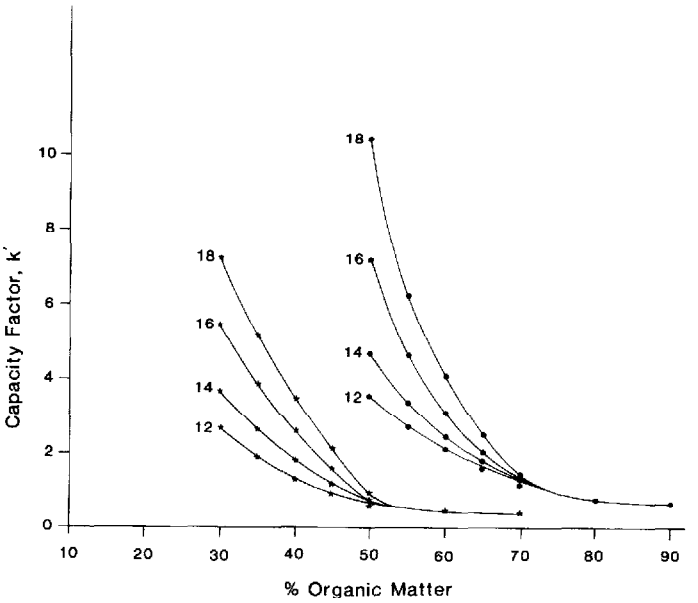

Fig. 2. Effects of % organic modifiers on k' values of ABDAC homologues. Organic modifiers: acetonitrile (★), methanol (●).

vious studies[10] that methanol tends to yield higher efficiency resolution of a mixture of the isomers than acetonitrile. This is in contrast to what is normally observed in traditional reversed-phase HPLC. As seen from the table, both resolution and selectivity characterized by R and α are dramatically affected by the change in the composition of a mobile phase. Thus, a reduction in the amount of water present in the mobile phase causes an appreciable decrease in R and α values. The decrease in R values may be due to a decrease in both α and k'. A decrease in α values with a reduction of water content in the eluent has also been observed generally in reversed-phase HPLC of homologous series[17].

Since the N-nitroso-alkylmethylamines (NAMA) in the homologous series shown in Fig. 3 are by far the most sensitive thermal energy analysis (TEA) detectants among known ABDAC-derived compounds used in indirect GC or HPLC analyses of parent ABDAC[2,14], we deemed it worthwhile to briefly study the chromatographic behavior of the NAMA series along with the ABDAC counterparts. Under identical HPLC conditions, the separation of an NAMA mixture (Fig. 3) was always better than that of the corresponding ABDAC components. Although not shown in Fig. 3B, baseline resolution of NAMA components was also achieved by using a mobile phase consisting of methanol–water (65:35). The differences in the magnitude of R values and α values determined under the same conditions for both series of compounds were noteworthy. With reference to Fig. 3, the respective R_{12-14}, R_{14-16}, R_{16-18}, α_{12-14}, α_{14-16}, and α_{16-18} estimated for the component peaks on chromatogram A are 1.11, 1.29, 1.10, 1.45, 1.69, and 1.50; and for those on chromatogram B are 0.85, 1.00, 1.22, 1.22, 1.18, and 1.23. The corresponding values for the ABDAC homologues are relatively lower (Table I). The dissimilar chromatographic outcome

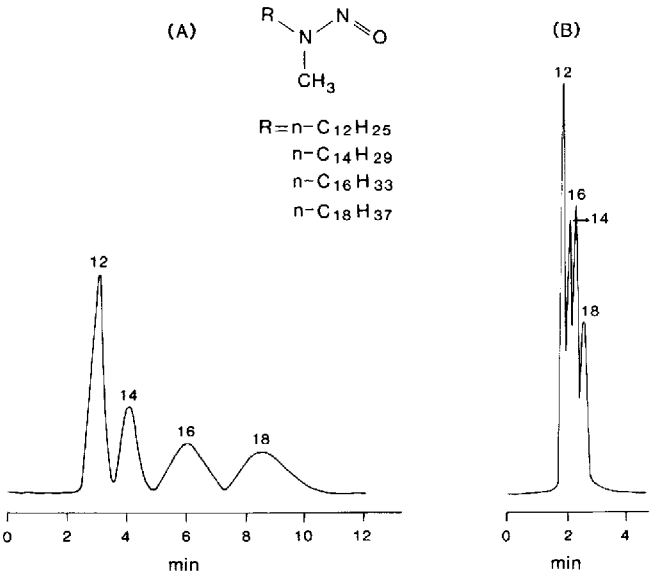

Fig. 3. Chromatographic separation of 12-, 14-, 16-, and 18-NAMA homologues on cyclodextrin-bonded silica. Mobile phases: (A) acetonitrile–water (40:60), (B) methanol–water (70:30).

of the two series may reflect the differences in molecular hydrophobicity and polarity between the two homologous series. In previous work[2], we demonstrated the separation of configurational (*E* and *Z* forms) isomers of each NAMA homologue by normal-phase HPLC. However, when current cyclodextrin methodology was used, we were unable to resolve the *E*–*Z* pair under all aqueous mobile phase conditions attempted. The result seems somewhat unusual in view of many literature reports that claim successful resolution of a wide variety of isomeric compounds with the cyclodextrin-bonded silica column.

For conventional reversed-phase HPLC of ionic compounds, theoretical treatment of solute partition mechanisms based on ion-pair, ion-exchange, and ion-interaction models has been advanced[18] to accommodate existing retention data for both anions and cations. Customarily, counter ions derived from organic or inorganic salts are added to mobile phases in all separations involving ionic species to be analyzed. Hence, it was logical to study the effects of mobile phase electrolytes on the chromatographic behavior of ABDAC cations. Fig. 4 provides representative chromatograms showing the separation of ABDAC homologues under the influence of mobile phase counter-ions. Interestingly, with argentation of the mobile phase (Fig. 4A), the mixture of ABDAC was unexpectedly well resolved into the four homologous components. While the origin of complexation is unknown and this argentation effect remains subtle, argentation techniques have been useful in numerous separations of geometrical isomers by virtue of the high complexation potential of the silver ion toward a donor substrate[19–22]. It must be pointed out that injection of ABDAC samples into mobile phases containing silver ions should lead to precipitation of silver chloride. This can impair the normal performance of the column resulting in poor separation of components. For practical application we recommend conversion of ABDAC into nitrate salts by an ion-exchange technique before injection of the

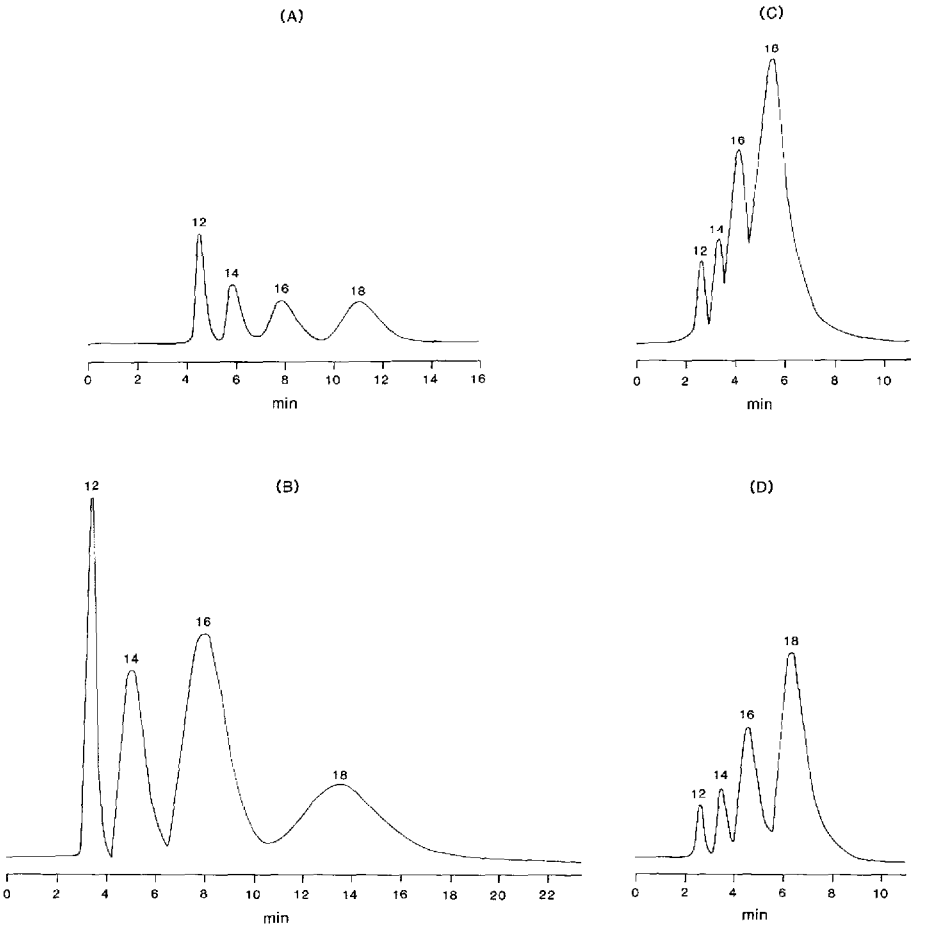

Fig. 4. Chromatographic separation of 12-, 14-, 16-, and 18-ABDAC homologues on cyclodextrin-bonded silica. HPLC conditions: all mobile phases consisted of acetonitrile–water (30:70) and electrolytes (0.1 *M*, pH 3.5); (A) silver nitrate, (B) sodium perchlorate, (C) sodium dihydrogen phosphate, (D) sodium methanesulfonate.

samples into HPLC systems. From our experience with ion-pair extractions of ABDAC using phase transfer techniques[3], the use of sodium perchlorate as a counter ion reagent for the quantitative recovery of quaternary ammonium cations from aqueous media or tissue samples met with much success. In consonance with this finding, HPLC with the mobile phase containing sodium perchlorate (Fig. 4B) revealed that each of the ABDAC components in this particular instance was also considerably more retainable by the stationary phase than in all other experiments executed under comparable conditions (Fig. 1B and Table II). It is plausible that ion-pair participation in the inclusion complex formation is responsible for the unusual retentivity, selectivity, and resolution capability observed in the latter case (Table II). On the other hand, retention of ABDAC homologues in the presence of other counter ions appears to follow mechanistically different pathways as each of these ammonium analytes showed less tendency to be retained (lower k' value) by

TABLE II

EFFECTS OF COUNTER ION ELECTROLYTES ON THE SEPARATION OF ABDAC HOMOLOGUES

For R and α determinations, see Table I; mobile phase flow-rate: 2.5 ml/min; each mobile phase contained 0.1 M counter ion electrolyte (pH 3).

Counter ion in water–acetonitrile (70:30)	*Component*									
	12-ABDAC			*14-ABDAC*			*16-ABDAC*			*18-ABDAC*
	k'	R	α	k'	R	α	k'	R	α	k'
Sodium perchlorate	2.32	1.13	(1.74)	4.03	1.20	(1.75)	7.04	1.86	(1.74)	12.2
Sodium methanesulfonate	1.61	1.38	(1.50)	2.41	1.00	(1.49)	3.60	1.29	(1.49)	5.35
Monobasic sodium phosphate	1.60	1.17	(1.39)	2.23	0.84	(1.39)	3.11	0.80	(1.40)	4.40
Sodium pentanesulfonate	1.77	0.51	(1.22)	2.16	0.59	(1.22)	2.64	0.60	(1.21)	3.20
Sodium octanesulfonate	1.21	0.00	(1.00)	1.21	0.00	(1.00)	1.21	0.00	(1.00)	1.21
Silver nitrate*	3.53	1.63	(1.39)	4.90	1.33	(1.40)	6.88	1.45	(1.40)	9.63
Sodium pentanesulfonate**	2.83	1.06	(1.46)	4.13	0.90	(1.46)	6.03	0.84	(1.46)	8.80

* Flow-rate at 1.5 ml/min.

** Mobile phase solvent composition: water–acetonitrile (75:25).

the column. We have previously described a similar trend of salt effects that was observed in reversed-phase HPLC of quinoidal imminium cations[23]. To demonstrate the counter ion effect on peak separation, two typical examples are given in Fig. 4C and D. Incorporation of sodium methanesulfonate into the mobile phase system is obviously more beneficial for the improved resolution of ABDAC components (Fig. 4D) than sodium phosphate (Fig. 4C). Further details of counter ion effects on k', R, and α parameters of ABDAC homologues are summarized in Table II. Close inspection of the data for alkane sulfonates in the homologous series shown in the table discloses that sodium methanesulfonate is the most satisfactory counter ion in the series as supported by the highest R and α values. The same data also indicate that the chromatographic characteristics obtained in the separation of ABDAC components are adversely affected by the presence of a higher member of the sulfonate homologues in the mobile phase. For example, with sodium octanesulfonate as the counter ion, HPLC of an ABDAC mixture yielded only a broad peak ($R=0$, $\alpha=1$). Significant improvement in the magnitude of R and α values can often be achieved by increasing the percentage of water in the mobile phase, as noticed in the case of sodium pentanesulfonate (Table II). Another feature related to the counter ion effects in question is that k' values for the higher members of the ABDAC homologous family are clearly more sensitive to the change in the homologous identity of alkanesulfonates present in the mobile phases.

Since the retention mechanism entailed in HPLC of ABDAC on cyclodextrin-bonded silica is uniquely different from that in traditional reversed-phase HPLC, it was desirable to see whether the capacity factor, k', of ABDAC would be a function of the alkyl chain length. We correlated the retention data from Table II and those from Table I according to the following equation:

$$\ln k' = aN + b$$

TABLE III

RELATION ($\ln k' = aN + b$) BETWEEN THE CAPACITY FACTOR, k', AND THE CARBON CHAIN LENGTH, N, IN ABDAC HOMOLOGUES

*HPLC conditions**	*Slope* a	*Intercept* b	$\ln k'_{10}$** *(calculated)*	$\ln k'_{10}$ *(found)*	r***
Acetonitrile–water (30:70)					
Sodium perchlorate	0.3014	−2.8755	0.2009	0.2015	0.997
Monobasic sodium phosphate	0.1657	−1.5127	0.1411	0.1423	0.996
Sodium methanesulfonate	0.1943	−1.8303	0.1013	0.1009	0.998
Sodium pentanesulfonate	0.1022	−0.5612	0.4105	0.4114	0.997
Sodium pentanesulfate	0.1986	−1.3006	0.5378	0.5383	0.997
Silver nitrate	0.1925	−1.0724	0.8466	0.8439	0.998
Acetonitrile–water (30:70)	0.1751	−1.1510	0.5894	0.5899	0.995
Methanol–water (50:50)	0.1993	−1.1018	0.8229	0.8235	0.999

* Concentration of each counter ion: 0.1 *M*.

** Intercept values at $N = 10$; k'_{10} = capacity factor of 10-ABDAC.

*** r = Correlation coefficient obtained from regression analysis. For mobile phases containing electrolytes, HPLC conditions are same as in Table II except for pH 3.3.

TABLE IV

DETERMINATION OF THE COMPOSITION OF VARIOUS ABDAC MIXTURES

A mobile phase of methanol–water (50:50) was used in all HPLC analysis. Sample Nos. 1, 2, and 3 were obtained by premixing various amounts of pure ABDAC components. Sample Nos. 4, 5, and 6 were from commercial sources. ND = None detected; TR = trace amount (less than 0.1%).

Component	*Composition (%) of sample No.*											
	1		*2*		*3*		*4*		*5*		*6*	
	HPLC	*MS*	*HPLC*	*MS*	*HPLC*	*MS*	*HPLC*	*MS*	*HPLC*	*MS*	*HPLC*	*MS*
12-ABDAC	19.30	19.52	9.45	9.60	7.04	7.00	4.00	3.93	40.11	40.30	ND	ND
14-ABDAC	23.55	23.70	51.78	51.93	8.56	8.74	93.05	92.87	49.96	50.41	TR	0.20
16-ABDAC	39.61	39.68	13.07	12.46	20.07	19.97	2.95	3.20	9.93	9.29	24.71	24.69
18-ABDAC	17.54	17.10	25.80	26.01	64.33	64.29	ND	ND	ND	ND	75.25	75.10

Without exception, the plots of ln k' *vs.* N (the number of carbons on the alkyl chain in ABDAC) were linear. Values of the slope (a) and the intercept (b) for each line were determined as given in Table III. The results of the linear correlation allow the combined use of the data in the equation and in the table to predict k' values for unknown components. The apparent parallelism between the results of this correlation study and those by conventional reversed-phase HPLC[2,24,25] suggests that in the present case, the total area of the hydrocarbonaceous alkyl chain in ABDAC is available for the inclusion complex formation.

To evaluate analytical applicability of the HPLC technique, we employed a mobile phase of methanol–water (50:50) for HPLC analysis of ABDAC standard solutions. Each standard solution was prepared by using a pure homologue obtained by preparative HPLC (see Experimental section) and calibration curves were obtained for all ABDAC homologues. Each curve that corresponded to a homologue indicated a linear region at concentrations ranging from 10 to 100 μg/ml. Detector responses for samples with concentrations beyond this region were not determined because samples of relatively high or low concentrations were rarely employed throughout this study. The precision of the measurements, expressed as relative standard deviations, averaged 3.67–6.61%. The calibration curves were used to determine the composition of unknown mixtures of ABDAC homologues. As shown in Table IV, the results from the HPLC method are in excellent agreement with those obtained by mass spectrometry (MS).

In conclusion, we have demonstrated first HPLC separation of quaternary ammonium homologues on a cyclodextrin-bonded stationary phase. Modification of chromatographic conditions by use of mobile-phase electrolytes is not always necessary in this system to effect separation. The method may be extended to other hydrocarbonaceous homologues including anionic analytes.

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