Technical

Separation of Surfactant Mixtures and Their Homologs by High Performance Liquid Chromatography

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ABSTRACT

A technique for separating similar and diverse ionogenic mixtures into their individual homologs was studied by high performance liquid chromatography on an octadecyl-silica (TSK Gel LS-410) column. Conditions used for the separation were: column size, 6 mm id × 200 mm, mobile phase, water/methanol (15:85, v/v) containing (a) 1.0 M sodium perchlorate and adjusting to pH 2.5 with phosphoric acid. (b) 0.1 M and pH 3.5; column temp., 50 C. By using 2 mobile phase conditions, various surfactant mixtures were separated into their individual homologs and simultaneously distinguished from each other, regardless of their ionogenic properties.

INTRODUCTION

Surfactants are classified as nonionic, anionic, cationic and amphoteric according to their ionogenic properties. Commercial toiletry and cosmetic products, e.g., shampoos and household detergents, usually contain 2 or 3 of the same or different ionogenic surfactants which are generally a mixture of homologs. As general properties of these commercial products are dependent on the combination of surfactants and lengths of the lipophilic groups, the simultaneous separation of each surfactant and that of their respective homologs is necessary to evaluate their properties.

Ion exchange chromatography (1) has been used to separate surfactant mixtures on the basis of their ionogenic difference. Separation of surfactants of like ionogenic character using salting-out chromatography was discussed in detail by Fudano et al. (2). However, the use of these column chromatographic techniques prolongs analysis, often taking days for separation. On the other hand, the homologs of individual surfactants have been separated by gas chromatography (GC). However, this method usually requires conversion of surfactants into volatile derivatives before analysis.

In recent years, several papers have been published on the analysis of surfactants using high performance liquid chromatography (HPLC) with porous microspherical poly(styrene-divinylbenzene) gels (3), and reverse phase packing containing octadecyl silane groups chemically bonded to silica gel (ODS/silica) (4-7). In these papers, however, emphasis is on the separation of individual surfactant homologs. Many difficulties are involved if they are to be applied to the analysis of surfactant mixtures, i.e., the simultaneous separation of individual surfactants from each other and from their respective homologs.

We have been studying the separation of surfactant homologs by HPLC using ODS/silica, which is the most widely used of the column packings, and reported 2 methods. One was the acid addition method using a mixture of water and methanol, adjusting to pH 2.2 with phosphoric acid as a mobile phase (8). The other was the salt addition method using a mixture of water and methanol containing 0.4 M sodium chloride (NaCl) (9). However, the separation of amphoteric surfactant homologs and the separation of individual surfactants from each other were not discussed in these papers.

In this study, a mixture of water, methanol, sodium perchlorate ($NaClO_4$) and phosphoric acid was used as the mobile phase to develop fundamental methodology applicable to the simultaneous separation of individual surfactants from each other and their respective homologs. As a result, 9 typical surfactants were separated into their respective homologs, regardless of their ionogenic properties. Moreover, these surfactant homolog mixtures were clearly distinguished from each other, by adjusting the mobile phase pH and the concentration of $NaClO_4$.

EXPERIMENTAL PROCEDURES

Materials

Homologs of 9 typical surfactants were used in this study. Their chemical structures and symbolic names used in this study are illustrated in Table I. The respective homologs were *n*-decyl, *n*-dodecyl, *n* tetradecyl, *n*-hexadecyl and *n*-octadecyl derivatives. The purities of alkyl chain length of all the surfactant homologs were > 95% by GC. FDE, BzAC, SAS and SNAG were the same grade as used in the previous studies. The ATC was special reagent-grade (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and was purified by recrystallization from ethanol. Other surfactant homologs were synthesized and purified in our laboratories by the techniques described later. Their chemical structures were confirmed by ^{13}C-nuclear magnetic resonance (NMR), ^{1}H NMR and infrared (IR) spectra. All solvents were of analytical reagent grade.

APC. A mixture of alkylchloride (0.01 M) and pyridine (0.01 M) was refluxed at 110-140 C for 3-4 hr. The reaction products were purified by recrystallization from acetone.

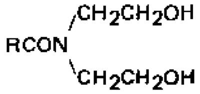
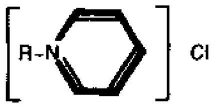
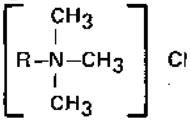
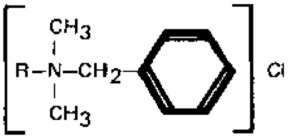
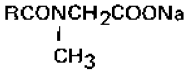
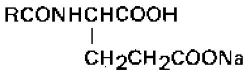
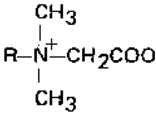
ADB. Alkyldimethylamine was prepared by reaction of alkylamine (0.1 M), formic acid (0.2 M) and formaldehyde (0.2 M) in the presence of 100 mL ethanol for 12 hr on a steam bath. In order to obtain alkyldimethylamine with an alkyl chain length of > 95% purity, the reaction products were fractionally distilled with a spinning-band column (Perkin-Elmer, USA).

Purified alkyldimethylamine (0.02 M) was allowed to react with sodium monochloroacetate (0.021 M) in the presence of 20 mL water and 20 mL isopropanol at 75 C for 5 hr. Water and isopropanol were then distilled off under reduced pressure. ADB and unreacted alkyldimethylamine were extracted with chloroform from the reaction products, and the latter was removed with petroleum ether by Soxhlet extraction.

SAP. Alkylamine (0.2 M) was dissolved in 100 mL cyclohexane. Methyl acrylate (0.22 M) was slowly added with stirring at 30-35 C followed by addition of 50 mL isopropanol. The reaction was done at 75-80 C for 2 hr. Unreacted methyl acrylate was distilled off under reduced

TABLE I

Chemical Structures of Surfactants Studied

Surfactant name	Symbolic name	Chemical structure
Nonionic surfactant		
(A) Fatty acid diethanolamide	FDE	 $RCON(CH_2CH_2OH)_2$
Cationic surfactant		
(B) Alkylpyridinium chloride	APC	
(C) Alkyltrimethylammonium chloride	ATC	 $[R{-}N(CH_3)_3]\,Cl$
(D) Alkylbenzyldimethylammonium chloride	BzAC	
Anionic surfactant		
(E) Sodium alkylsulfate	SAS	$ROSO_3Na$
(F) Sodium N-acylsarcosinate	SNAS	 $RCON(CH_3)CH_2COONa$
(G) Sodium N-acyl-L-glutamate	SNAG	 $RCONHCH(COOH)CH_2CH_2COONa$
Amphoteric surfactant		
(H) Alkyldimethylaminoacetic acid betaine	ADB	 $R{-}N^+(CH_3)_2{-}CH_2COO^-$
(I) Sodium alkylaminopropionate	SAP	$RNHCH_2CH_2COONa$

pressure. The reaction products were suspended in 100 mL water. Sodium hydroxide (0.2 M) was slowly added with stirring at 90 C. The solution was neutralized with 10% hydrochloric acid. Excess water was removed on a steam bath. Nonhydrolyzed methyl alkylaminopropionate was removed with petroleum ether by Soxhlet extraction. SAP was then extracted with ethanol. The extracted SAP was purified by recrystallization from ethanol.

HPLC Apparatus and Operating Condition

A Model ALC/GPC 201 liquid chromatograph (Waters Assoc., Milford, MA) equipped with a Waters Model R401 differential refractometer and a Waters Model 730 data module were used in this study.

The chromatographic column (6 mm id × 200 mm) was constructed of stainless steel. Warm water was run through the jacket to maintain the column temperature at 50 C. The column was packed with TSK Gel LS 410 (5 μ, spherically shaped ODS/silica: Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan) using a mixture of methanol, dioxane, carbon tetrachloride and "slurry solvent B" conc (prepared by Macherey-Nagel, Düren, West Germany) as a slurry-forming solvent. Chloroform was used as a pressurizing solvent (packing pressure: 450 kg/cm^2).

All the samples were prepared as 0.5-1.0% methanol solutions, and 10-20 μL were injected into the HPLC column using a Waters Model U6K septumless loop injector. All experiments were done under isocratic conditions. The flow rate of the mobile phase was set at 1.5 mL/min. Capacity factor (k') values and height equivalent to a theoretical plate (HETP) values were evaluated from the chromatograms in the usual way (10).

RESULTS AND DISCUSSION

Our previous study (9) revealed that the different inorganic salts and their concentrations, as well as the water content in the mobile phase affected the separation of the surfactant homologs in the salt addition method. In this study, the results indicate that not only these factors, but different organic solvents and mobile phase pH also affect the separation. These factors therefore necessitate that the conditions be optimized.

Ammonium chloride (NH_4Cl), $NaClO_4$ and NaCl were examined as additives. The volume ratio of water to methanol in the mobile phase was 15:85 whereas the concentration of the individual additive was 0.2 M. The k' values and the HETP values of the respective hexadecyl derivatives are given in Table II. The results indicated that these values varied with different inorganic salts. Generally, increase of the k' values resulted in good separation of homologs. In order to increase k' values, NaCl was effective for cationic and amphoteric surfactants whereas $NaClO_4$ was effective for nonionic and anionic surfactants. The HETP values of all the surfactants generally decreased and thus sharpness of each peak increased by using $NaClO_4$ as an additive. Instead of the NaCl used in the previous paper, $NaClO_4$ was chosen as the additive in consideration of the HETP values and solubility in the mobile phase used.

As a mixture of water and water-miscible organic sol-

TABLE II

Effect of Inorganic Salts on k′ and HETP

Surfactant[a]	NaCl k′	NaCl HETP (mm)	$NaClO_4$ k′	$NaClO_4$ HETP (mm)	NH_4Cl k′	NH_4Cl HETP (mm)
FDE	3.23	0.8×10^{-2}	3.54	0.7×10^{-2}	3.30	0.8×10^{-2}
APC	3.83	23.4	2.67	4.8	3.20	9.6
ATC	2.54	6.0	2.19	1.0	2.22	3.2
BzAC	3.16	1.6	2.52	1.0	2.73	0.9
SAS	3.60	1.1	4.47	0.8	3.39	1.1
SNAS	3.41	2.7	4.30	1.1	3.62	1.6
SNAG	2.18	1.2	2.79	1.0	2.28	0.8
ADB	3.16	3.8	2.61	2.6	2.67	3.0
SAP	2.71	2.4	2.59	8.4	2.39	1.4

[a] Hexadecyl derivatives.

vents is usually used as the mobile phase in reverse-phase chromatography, methanol and acetonitrile were examined in this study. Water content and the concentration of $NaClO_4$ were set at 10% and 0.2 M, respectively. The water/methanol mobile phase gave good separation for all of the surfactant homologs. However, water/acetonitrile gave poor chromatograms for several ionogenic surfactants such as ADB, SAP and SNAG, as shown in Figure 1. This phenomenon is attributed to the fact that these ionogenic surfactants have little solubility in acetonitrile. A mixture of water and methanol therefore was used as the mobile phase in this study.

The effect of $NaClO_4$ concentration on the k′ values of the respective hexadecyl derivatives is shown in Figure 2. In the concentration range 0.025-0.1 M, the k′ values of the nonionic and amphoteric surfactants were fairly constant, whereas those of the anionic and cationic surfactants increased slightly with increasing $NaClO_4$ concentration. The k′ values of all the surfactants increased markedly with increasing concentration of $NaClO_4$ in the range 0.1-1.0 M. As described in our previous paper, the remarkable increases in the k′ values were due to a salting-out effect. The homologous separation of all the surfactants increased and the HETP values decreased by increasing the concentration of $NaClO_4$.

At 3 concentrations of $NaClO_4$ (0.025, 0.1 and 1.0 M), the effect of the mobile phase pH on the k′ values was examined by adding phosphoric acid to the mobile phase. The volume ratio of water/methanol was 15:85. The results obtained on the hexadecyl derivatives are shown in Figure 3. These results indicate that the effect of the mobile phase pH on the k′ values are dependent on both the ionogenic properties of surfactants and the concentration of $NaClO_4$. The mobile phase pH had little effect on the k′ values of nonionic and cationic surfactants. In the case of anionic surfactants, the k′ values increased by increasing the acidity of the mobile phase. This effect was most remarkable at the 0.025 M $NaClO_4$ concentration. Amphoteric surfactants behaved in an interesting manner. Their k′ values were independent of the mobile phase pH in 1.0 M $NaClO_4$. At 0.025 and 0.1 M $NaClO_4$, the k′ values decreased drastically by increasing the acidity of the mobile phase in the pH range 2.5-4.0. This behavior is attributed to the electrostatic properties of amphoteric surfactants which act as cationic surfactants in an acidic environment. As the concentration of $NaClO_4$ was decreased, the effect of the mobile phase pH on the k′ values of all the surfactants was also decreased. From these results, it was concluded that the ion supression effect of $NaClO_4$ was stronger than that of phosphoric acid. On the other hand, the HETP values were generally decreased by increasing the concentration

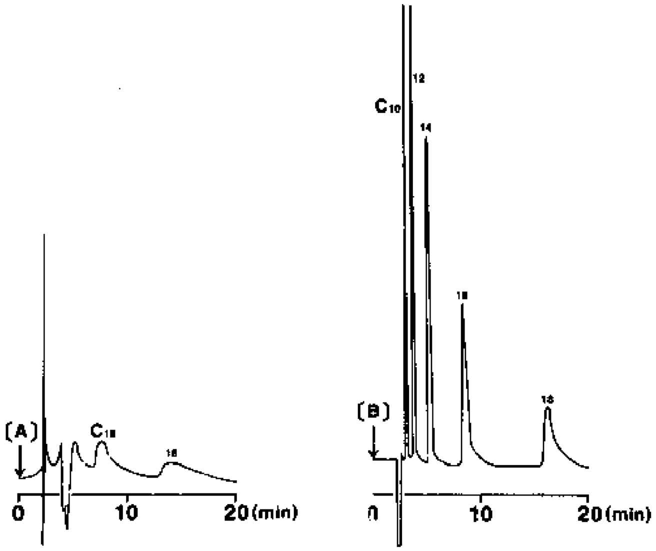


FIG. 1. Effect of organic solvent on separation. (A): water/acetonitrile; (B): water/methanol; sample: ADB homologs, e.g., C_{10} means alkyl chain length in lipophilic group is 10.

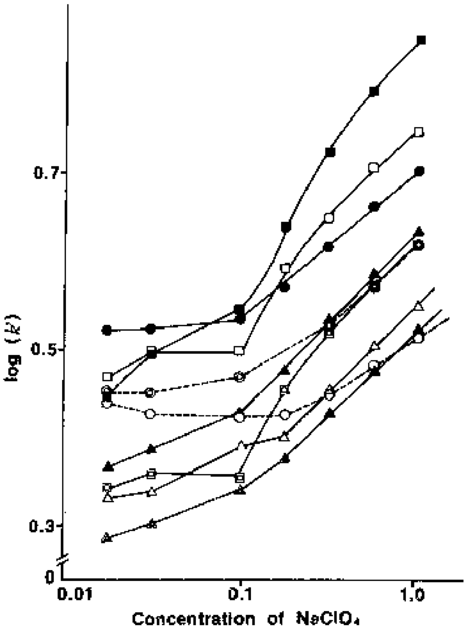


FIG. 2. Effect of $NaClO_4$ concentration on k′. ●=FDE, ⍋=ATC, △=APC, ▲=BzAC, ■=SAS, □=SNAS, ⊡=SNAG, ○=ADB and ◎=SAP. All the surfactants are hexadecyl derivatives.

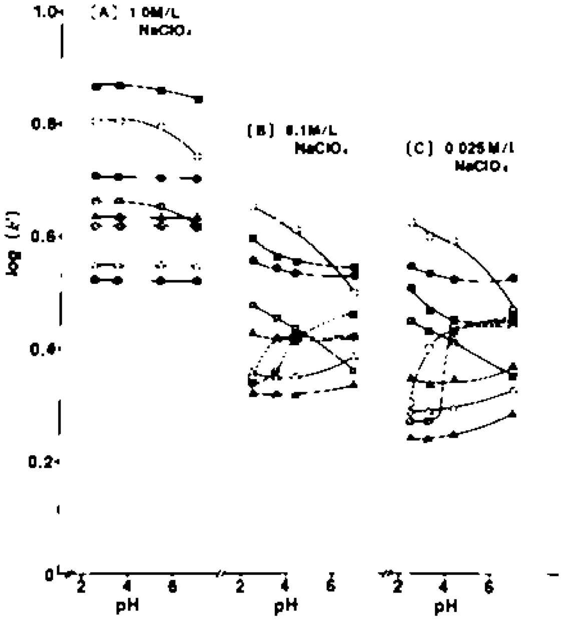


FIG. 3. Effect of mobile phase pH on k'. Symbols are the same as in Fig. 2.

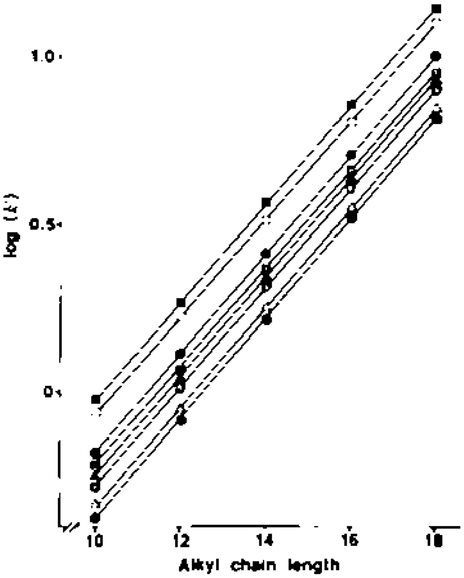


FIG. 4. Relationship between log (k') and alkyl chain length in lipophilic group. Symbols are the same as in Fig. 2.

of $NaClO_4$ and acidity of the mobile phase. Therefore, the concentration of $NaClO_4$ and the mobile phase pH were set at 1.0 M and 2.5, respectively, in consideration of the HETP values and the acid resistance limit of ODS/silica.

As the water content in the mobile phase was increased, the k' values increased, which resulted in improved separation of the homologs. This water content effect was the same as that described in the previous paper. In this study, the volume ratio of water to methanol was set at 15:85.

Figure 4 shows the relationship between the lipophilic group alkyl chain length and logarithmic k' values using the mobile phase just given. A linear relationship was found for all the surfactant homologs. As shown in Figure 4, all the surfactants used in this study were efficiently separated into their respective homologs isocratically. Figure 4 also indicates that various ionogenic surfactant mixtures are simultaneously separated into their individual homologs. Typical separations of surfactant mixtures are shown in Figure 5 (one nonionic and 2 amphoteric surfactants), Figure 6 (3 anionic surfactants) and Figure 7 (3 cationic surfactants), respectively. A mixture of anionic (SAS), amphoteric (ADB) and nonionic (FDE) surfactant homologs was also separated into their individual homologs as shown in Figure 8.

The reproducibility and degree of quantitation obtained by the present method was examined. In the case of the mixture of APC-SAP homologs and SAS-FDE homologs, the analytical results are summarized in Table III. Good reproducibility was obtained; Cv (%) was equal to or lower than 2.91%. Thus, the present technique was proven to be convenient in separating mixtures of the same or different ionogenic surfactants into their individual homologs in one chromatographic analysis.

Two combinations of surfactant homologs were not separated using this same mobile phase. One was a mixture of ADB and TMC homologs. Their corresponding homologs had the same k' values as shown in Figure 4. By using the characteristic dependence of ADB on the mobile phase pH and the concentration of $NaClO_4$, they were efficiently separated into their respective homologs and clearly distinguished from each other as shown in Figure 9. The mobile phase was adjusted to 0.1 M $NaClO_4$ and pH 3.5. The other was a mixture of SNAG and ADB homologs. The homologs differed in alkyl chain length by 2 methylene units, but sodium N-tetradecylsarcosinate and hexadecyldi-

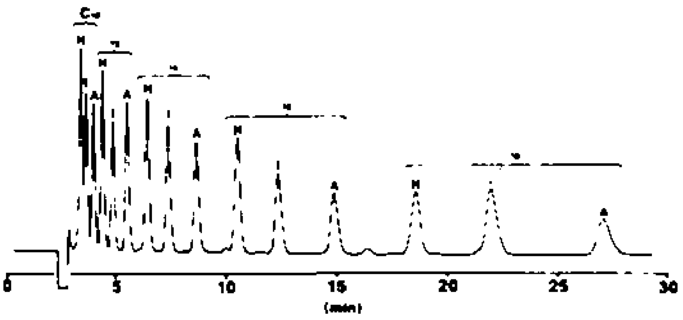


FIG. 5. Separation of a mixture of FDE(A), ADB(H) and SAP(I) homologs. Mobile phase: water/methanol (15:85, v/v) containing 1.0 M/L $NaClO_4$ and adjusting to pH 2.5 with phosphoric acid.

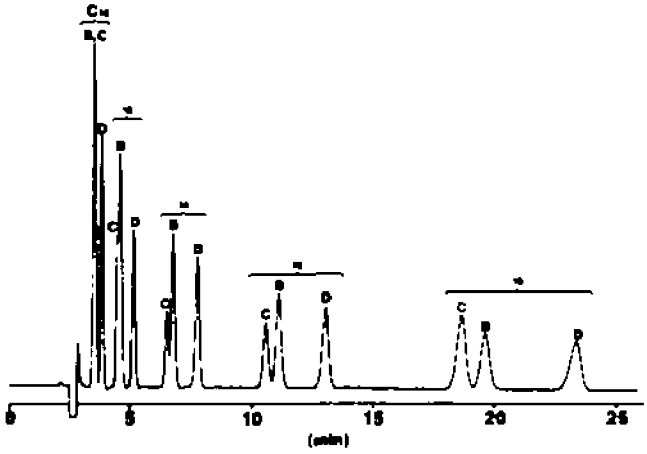


FIG. 6. Separation of a mixture of APC(B), ATC(C) and BzAC(D) homologs. Mobile phase condition is the same as in Fig. 5.

methylaminoacetic acid betaine had the same k′ values. This mixture was separated with 0.1 M $NaClO_4$ and pH 3.5-adjusted mobile phase.

As the combination of these particular anionic and cationic surfactants is unusual in commercial products, they were not discussed in this study.

It is possible to apply this newly developed technique to the separation and identification of many other ionogenic surfactant homologs. Recently, 15 dodecyl derivatives (4 nonionic, 7 anionic and 4 amphoteric) used in commercial shampoos were found to be efficiently separated and clearly distinguished from each other by this technique. These analytical results will be reported in our next paper.

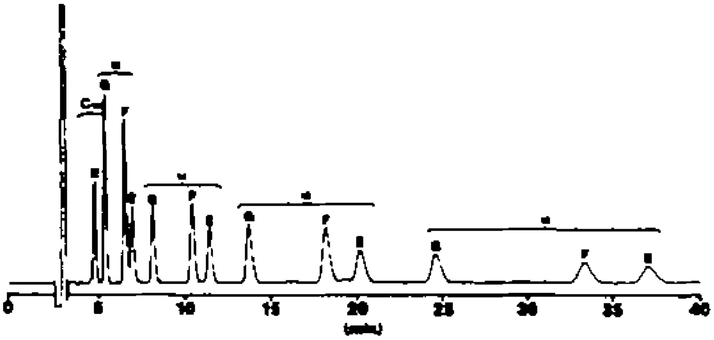


FIG. 7. Separation of a mixture of SAS(E), SNAS(F) and SNAG(G) homologs. Mobile phase condition is the same as in Fig. 5.

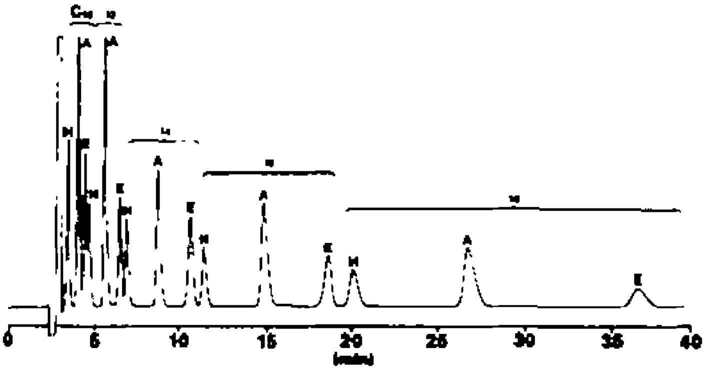


FIG. 8. Separation of a mixture of FDE(A), SAS(E) and ADB(H) homologs. Mobile phase condition is the same as in Fig. 5.

TABLE III

Reproducibility of Homologous Distribution

Sample	Alkyl chain length	Present (mol %)	Found[a] (area %)	Cv[b] (%)
Mixture (A)				
APC	10	22.63	22.09	0.51
	12	20.01	19.85	0.38
	14	18.56	17.82	0.40
	16	20.64	21.68	0.58
	18	18.16	18.56	0.30
SAP	10	20.59	20.10	2.10
	12	17.34	17.71	1.25
	14	19.47	19.17	1.00
	16	19.33	19.70	1.28
	18	23.27	23.36	2.74
Mixture (B)				
SAS	10	20.58	19.76	0.75
	12	22.77	21.34	2.47
	14	18.93	19.61	0.83
	16	18.40	19.87	2.63
	18	19.32	19.41	2.91
FDE	10	24.66	24.27	1.68
	12	21.22	21.04	0.84
	14	22.16	23.37	1.06
	16	15.54	14.94	0.89
	18	16.42	16.38	2.06

[a] Taken as the average of 5 replicate analyses.

[b] Coefficient of variation.

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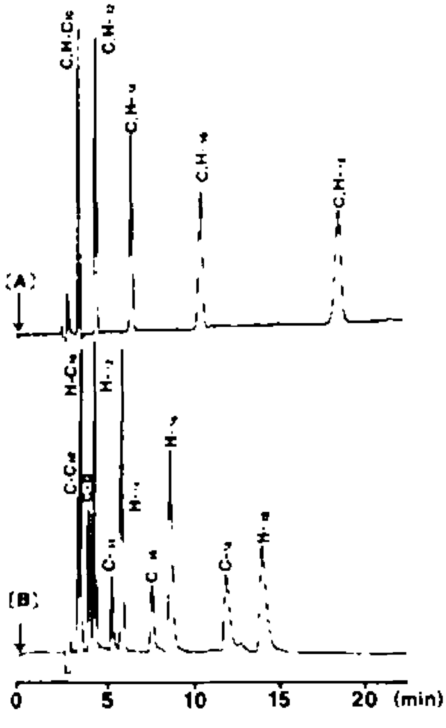

FIG. 9. Separation of a mixture of ATC(C) and ADB(H) homologs. (A): mobile phase condition is the same as in Fig. 5; (B): mobile phase is water/methanol (15:85, v/v) containing 0.1 M/L $NaClO_4$ and adjusting to pH 3.5.