Forensic Toxicological Determination of Surfactant by Liquid Chromatography/ Electrospray Ionization Mass Spectrometry and Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry

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A procedure for identifying surfactants was investigated by liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) and liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) from the forensic toxicological point of view. The analysis of anionic, cationic, and nonionic surfactants in both negative and positive mode revealed that anionic and cationic surfactants are detected as M^- ions in the negative mode and M^+ ions in the positive mode, respectively, while $[M+H]^+$ ions or $[M+NH_4]^+$ ions appeared for nonionic surfactants in the positive mode. Anionic surfactants (linear alkylbenzene sulfonate and polyoxyethylene alkyl ether sulfate) were successfully extracted using a weak anion exchange cartridge, with recovery rates of 53.8–76.7%. Cationic surfactant (benzalkonium) was also easily extracted using a weak cation exchange cartridge with a recovery rate of 107%. When aqueous solutions of cationic, anionic, and nonionic surfactants were concentrated to dryness, the recovery rates ranged from 65.8% to 124%. By analyzing anionic surfactants (alpha-olefine sulfate, polyoxyethylene alkyl ether sulfate, and linear alkylbenzene sulfonate) in the product ion scan mode in LC/ESI-MS/MS, it was possible to identify the structure of the hydrophilic group of surfactants, and MS/MS was confirmed to be a powerful technique for classification.

Key words —— surfactant, poisoning, liquid chromatography/mass spectrometry, electrospray ionization, liquid chromatography/electrospray ionization tandem mass spectrometry

INTRODUCTION

Many cases of poisoning occurred recently in Japan, with numerous instances of mixing detergents into food and beverages. Detergent is an item usually found in households for dish washing, laundry, bleaching, *etc*. It is therefore easy for criminals to access these poisonous substances for such purposes as injuring others, malicious mischief, and faking injury to themselves. Although surfactants, the main components of detergents, are associated with many environmental problems, their relative lack of acute toxicity has not drawn much serious attention from forensic toxicologists. When investigating cases of surfactants poisoning, detection and identification of surfactants is of great significance. An procedure for identifying of surfactants is thus required in forensic fields to specify their ionicity, structure, or compositions, although the extremely large variety of components and detergent products make the determination of surfactants an intricate task.

The determination of surfactants has so far been performed using the color test with ion-association reagents,[1] high-performance liquid chromatography (HPLC),[2–4] *etc*. As the information acquired with these conventional analytical methods is very limited, their complete structures, molecular mass, or degree of polymerization of ethyleneoxide (EO) has been difficult to obtain. Gas chromatography/mass spectrometry (GC/MS) analysis of surfactants requires tedious pretreatment processes such as derivatization.[5] On the contrary, liquid chromatography/mass spectrometry (LC/MS), which has recently been increasingly applied to determine surfactants,[6–11] allows the detection of polar substances

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without such tedious pretreatment steps as derivatization. The authors have already successfully applied the LC/MS methodology for the detection of ionic or highly polar compounds including muscle relaxants[12,13] and drug glucuronides.[14–16] Electrospray ionization (ESI) is especially useful for analyzing ionic compounds, i.e., surfactants, and it is easy to obtain their molecular masses. LC/ESI-MS permits chromatographic separation of mixtures, detection of compounds lacking remarked UV absorption, specification of the number of EO and structure of the alkyl moiety, identification of its group, and further discrimination between commercially available detergent products. This makes the method one of the most useful tools for investigating cases of poisoning with surfactants.

In the present study, to develop a quick and sensitive LC/MS procedure that allows the detection of surfactants without tedious pretreatment steps, both chromatographic and instrumental parameters were optimized, and the procedure of structural identification from the mass spectra of various surfactants was also examined.

MATERIALS AND METHODS

Chemicals —— Standard surfactants were kindly provided by Kao Corporation (Tokyo, Japan), Lion Corporation (Tokyo, Japan), and P&G (Kobe, Japan). Benzalkonium (BZ) chloride was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Standard stock solutions of these compounds (2%, w/v) were prepared in ultrapure water or methanol (HPLC grade). Spiked samples were prepared by adding the standard solution to ultrapure water or beverages immediately prior to analysis. Methanol was of HPLC grade, and other chemicals were of analytical grade. Solid-phase extraction cartridges (Bond Elut® CBA and Bond Elut® PSA) were purchased from Varian (Harbor City, CA, U.S.A.).

Instrumentation —— LC/ESI-MS was performed on a platform (Micromass, Manchester, U.K.) mass spectrometer equipped with an ESI interface linked to two PU-980 HPLC pumps (JASCO Corporation, Tokyo, Japan) in negative mode for anionic surfactants and positive mode for cationic and nonionic surfactants. Cross-flow interface as an ion source was set at 100°C. Capillary and cone voltages were set at –3.0 kV and –20 V for negative mode and 3.5 kV and 20 V for positive mode, respectively.

LC/ESI-MS/MS was carried out on a Micromass Quattro LC triple-stage quadrupole mass spectrometer equipped with an ESI interface linked to an 1100 HPLC pump (Agilent Technologies, Palo Alto, CA, U.S.A.). Ion source temperature and capillary and cone voltages were set at 100°C, –3.0 kV and –30 V, respectively. Collision-induced dissociation (CID) was accomplished at an argon gas pressure of 2.3×10^{-3} mbar and a collision energy of 20 or 30 eV.

The LC columns used were an L-column ODS (1.5 mm i.d. × 150 mm, Chemicals Evaluation and Research Institute, Tokyo, Japan) and an Asahipak GF-310HQ (2.0 mm i.d. × 150 mm, Showa Denko K.K., Tokyo, Japan). A gradient mobile phase system, composed of eluent A [methanol-ammonium acetate 10 mM (50 : 50, v/v)] and eluent B [methanol-ammonium acetate 10 mM (95 : 5, v/v)], was applied to the L-column ODS column; the concentration of eluent B was linearly increased from 40% to 100% (3%/min) and then maintained for 30 min. For the Asahipak GF-310HQ column, methanol-ammonium acetate 10 mM (50 : 50, v/v or 75 : 25, v/v) were used as mobile phases. The flow rate in each analysis was 0.1 ml/min.

All samples were filtered through a 45-μm membrane filter (GL Sciences Inc., Tokyo, Japan) before injection.

RESULTS AND DISCUSSION

Mass Spectra of Anionic, Cationic, and Nonionic Surfactants

Surfactants were generally classified into four ionic types character: anionic; cationic; nonionic; and amphoteric (betaine). In this study, we selected the 10 major anionic, cationic, and nonionic surfactants (Fig. 1) that are frequently used in household detergents, and their mass spectra were measured by flow-injection LC/ESI-MS in negative and positive modes.

In every mass spectrum of anionic surfactants, linear alkylbenzene sulfonate (LAS) and alkylsulfuric ester (AS) negatively charged molecular ions (M^- ions) were observed in the negative mode, as shown in Fig. 2. In the spectrum of LAS, plural M^- ions appeared (Fig. 2A), because it was a mixture of compounds that have four different alkyl groups (C_{10}–C_{13}). C_{10}–, C_{11}–, C_{12}–, and C_{13}–LAS exhibited predominant M^- ions at *m/z* 297, 311, 325, and 339, respectively. Each M^- ion was separated by 14 mass units, corresponding to one methylene (–CH_2–). In the spectrum of AS (Fig. 2B), three M^-

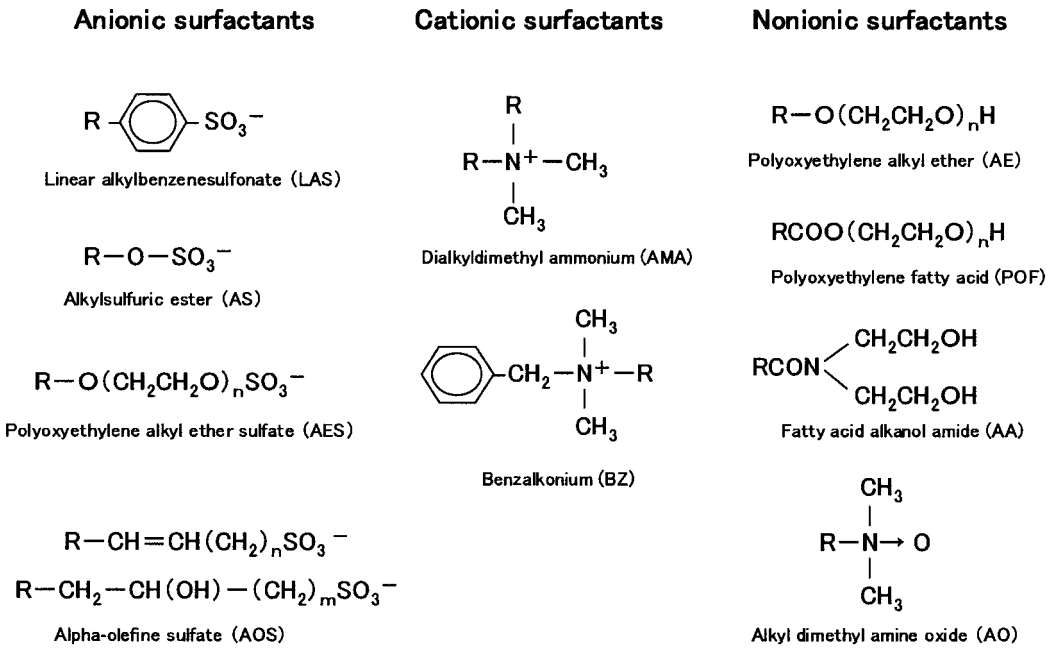

Fig. 1. Structures of 10 Major Anionic, Cationic, and Nonionic Surfactants

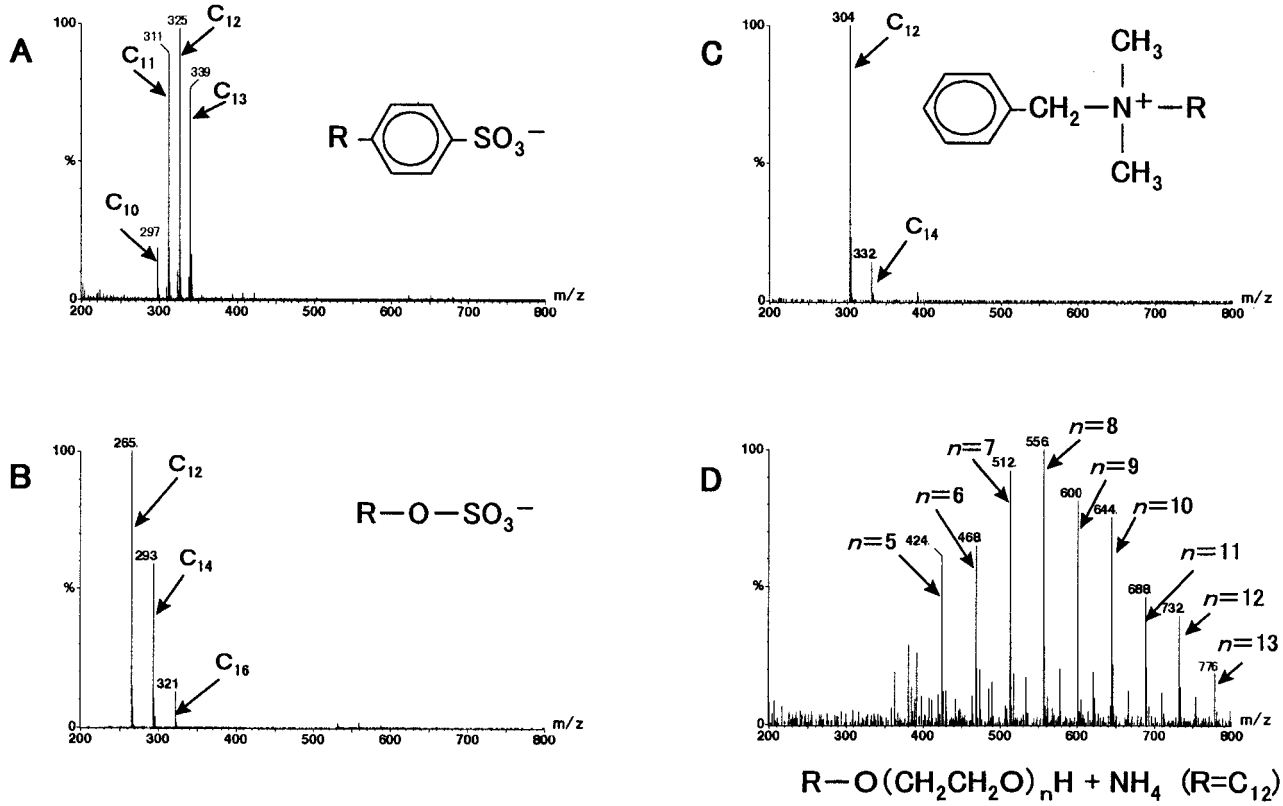

Fig. 2. Mass Spectra of Linear Alkylbenzenesulfonate (A), Alkylsulfuric ester (B), Benzalkonium (C), and Polyoxyethylene Alkyl Ether (D) by Flow Injection Mode

A and B were measured in negative mode, and C and D in positive mode.

ions at *m/z* 265, 293, and 321 were also observed and assigned to C_{12}–, C_{14}–, and C_{16}–AS, respectively. These ions are separated by 28 mass units, corresponding to the repeat unit of the ethylene chain (–C_2H_4–). It is well known that fatty acids found in natural products have even-numbered carbon alkyl chains. Surfactants such as AS (Fig. 2B) are therefore believed to have originated from animal or plant fat, while surfactants such as LAS (Fig. 2A) were manufactured from synthetic fatty acids. In addition, some M^- ions were also obtained from other anionic surfactants, i.e., polyoxyethylene alkyl ether sulfate (AES) and alpha-olefine sulfate (AOS).

BZ, a cationic surfactant, showed a molecular (M^+) ion in positive mode (Fig. 2C). The constituent ions at *m/z* 304 and 332 were assigned to C_{12}–BZ and C_{14}–BZ, respectively. According to the *Pharmacopoeia of Japan*, BZ chloride includes 40% or more of C_{12}–BZ and 70% or more of a mixture of C_{12}– and C_{14}–BZ. The mass spectrum in Fig. 2C demonstrates that C_{12} and C_{14} compounds are the basis of this surfactant. Similarly, M^+ ions were obtained in the mass spectrum of dialkyldimethyl ammonium (AMA).

Polyoxyethylene alkyl ether (AE) is one of the most frequently used nonionic surfactants, and its mass spectrum (Fig. 2D) showed the characteristic distribution of the main peaks that are separated by 44 mass units in positive mode. The separation of 44 mass units reflects a difference in the degree of polymerization EO (–CH_2CH_2O–). The mass spectra of polyoxyethylene-related surfactants showed a very similar distribution of the main peaks. As shown in Fig. 2D, the mass spectrum of C_{12}–AE gave the ammonium-adducted molecule ($[M+NH_4]^+$) of each ingredient instead of $[M+H]^+$ ions, and the mass spectra of other AEs were also characterized by $[M+NH_4]^+$ ions with the same pattern due to their low proton affinities. Additionally, as shown in Fig. 2D, degree of polymerization of EO of the most intense ion at *m/z* 556 was calculated to be 8, and approximately 8 compounds were further confirmed in the spectrum. For other nonionic surfactants, including polyoxyethylene fatty acid ester (POF), fatty acid alkanol amide (AA), and alkyl dimethyl amine oxide (AO), $[M+H]^+$ ions instead of $[M+NH_4]^+$ ions were observed. A protonated dimer ($[2M+H]^+$) was further obtained for AO, as was also observed for dimethylamphetamine-*N*-oxide.[17] This demonstrates the existence of the *N*-oxide moiety.

These findings indicate that the negative mode is useful in the identification of anionic surfactants, while the positive mode is advantageous for detecting cationic and nonionic surfactants. Negatively or positively charged molecules (M^- or M^+) appear in the mass spectra of anionic and cationic surfactants, whereas $[M+H]^+$ or $[M+NH_4]^+$ ions should be monitored for nonionic surfactants. Takino *et al.*[7] also detected $[M+NH_4]^+$ ion for one nonionic surfactants, a nonylphenol ethoxylate oligomer. Eichhorn and Knepper[8] also reported that the amphoteric surfactant cocamidopropylbetaine gave $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ ions in the positive mode and $[M-H]^-$ ions in the negative mode. The present study revealed that anionic surfactants give their M^- ions only in the negative mode, and that cationic and nonionic surfactants give only their molecular-related ions such as M^+, $[M+H]^+$, and $[M+NH_4]^+$ in the positive mode.

The detection limits in the full scan mode ranged from 0.05 ppm (for nearly single-component surfactants such as BZ) to 1 ppm (for multi component surfactants such as AE).

Optimization of Chromatographic Conditions

The 10 surfactants listed in Fig. 1 were analyzed on a semi micro L-column ODS with a gradient mobile-phase system. Most surfactants, with the exception of AE and AMA, were successfully detected with excellent separation in 50 min. AE contains many components, which have extensive polymerization of ethoxylate, and co-eluted at the same retention time under these conditions, as shown in Fig. 3A. The ingredients of AMA were strongly retained on the L-column ODS because of its low polarity. The peak shape of $2C_{16}$–AMA was not good, and $2C_{17}$– and $2C_{18}$–AMA were not fully eluted (Fig. 3C).

On the other hand, using a semi micro GF-310HQ column for gel permeation chromatography (GPC) with a mobile phase of methanol-ammonium acetate 10 mM (50 : 50, v/v), good separation was obtained for the components of AE (Fig. 3B). It is known that the GPC-type column excludes ionic compounds under such a low salt concentrations of the mobile phase as ammonium acetate 10 mM. Anionic or cationic surfactants would scarcely be retained on the GPC column although the components of AE eluted in order of decreasing molecular weight with good separation due to their nonionic properties, conforming the GPC separation mode. In addition, AMA was more efficiently analyzed on the GPC column with a mobile phase of methanol-ammonium acetate 10 mM (75 : 25, v/v) than on the

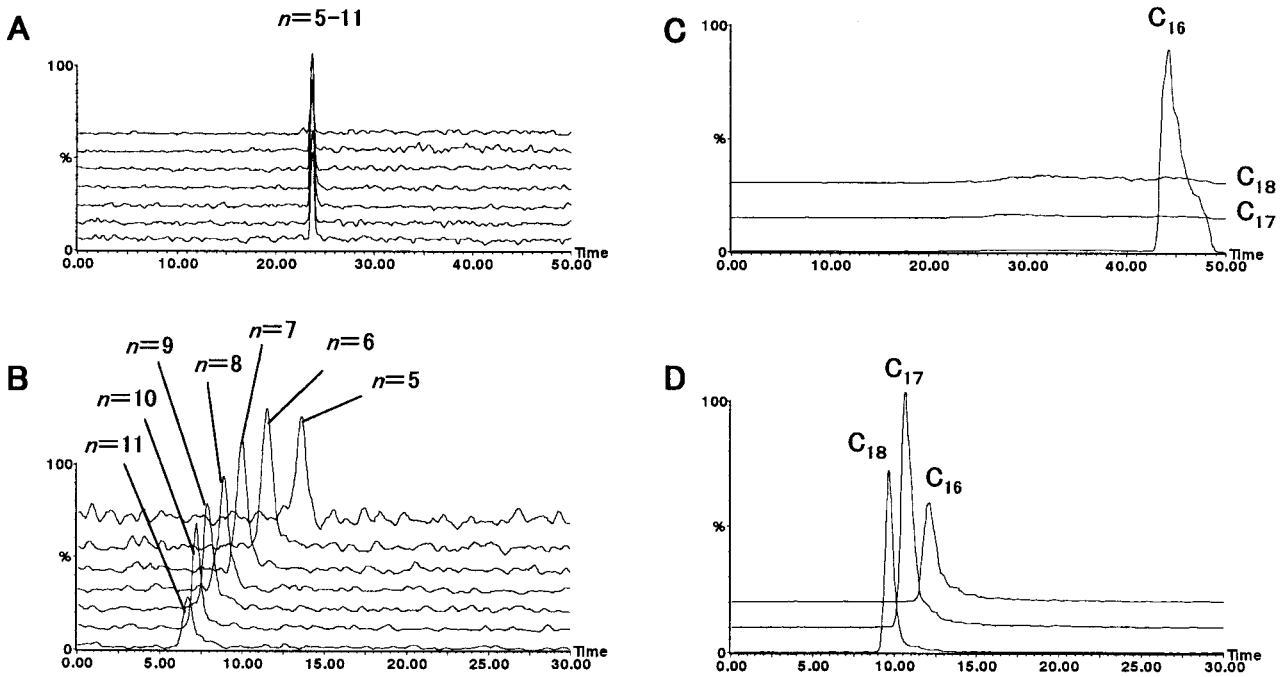

Fig. 3. The Mass Chromatograms Obtain for Polyoxyethylene Alkyl Ether (A, B) and Dialkyldimethyl Ammonium (C, D)

Chromatographic conditions for A and C, B, and D were gradients of methanol and ammonium acetate 10 mM (the concentration of methanol is increased from 68% to 95%) on L-column ODS, methanol-ammonium acetate 10 mM (50 : 50, v/v) on Asahipak GF-310HQ, and methanol- ammonium acetate 10 mM (75 : 25, v/v) on Asahipak GF-310HQ, at flow rates of 0.1 ml/min, respectively.

ODS column. As these compounds eluted in the order of lower molecular weight, a separation mode for example, hydrophobic interaction, could function along with the GPC mode. Based on these results, we primarily employed the L-column ODS with a gradient mobile-phase system for analysis of surfactants, except that GF-310HQ was used for the analysis of AE and AMA together with L-column ODS.

Identification of Alkyl Groups of Various AEs from Mass Spectra

Anionic and cationic surfactants give M^+ or M^- ions, and the main ions in each mass spectrum reflect the molecular weights of surfactants. This allows easy identification of their molecular weights and chemical structures. On the other hand, nonionic surfactants give $[M+H]^+$ or $[M+NH_4]^+$ ion in the positive mode, which does not allow such easily identification of their molecular weights and structures. Due to their large varieties of alkyl groups and diversity of EO polymerization, numerous AE products are on the market. Identifying the structure of AE is therefore especially important but difficult.

For identification of the alkyl group, three types of AE for which the alkyl group was known were analyzed by flow-injection mode. Figures 4A, 4B, and 4C illustrates the mass spectra of $C_{12=0}$–AE ($R = C_{12=0}$), $C_{16=0}$–AE ($R = C_{16=0}$), and $C_{18=1}$–AE ($R = C_{18=1}$) of which the alkyl groups are derived from lauric acid, palmitic acid, and oleic acid, respectively. Each component gave $[M+NH_4]^+$ ions, and all spectra showed the characteristic distribution of the main peaks that are separated by 44 mass units, reflecting the polymerization degree of EO. Examples of components having six degrees of polymerization of EO ($n = 6$) are shown in Fig. 4. Ions at m/z 468 for $C_{12=0}$–AE, m/z 524 for $C_{16=0}$–AE, and m/z 550 for $C_{18=1}$–AE correspond to their $[M+NH_4]^+$ ions. These typical spectra reflect the AE alkyl group. The separation of 56 mass units between ions at m/z 524 of $C_{16=0}$–AE and m/z 468 of $C_{12=0}$–AE was confirmed to correspond to the butylene chain ($-C_4H_8-$). Similarly, the separation of 26 mass units between ions at m/z 524 of $C_{16=0}$–AE and m/z 550 of $C_{18=1}$–AE derived from the ethyne chain ($-C_2H_2-$). Thus close study of the structures of the alkyl groups by examination of their mass spectra is effective in identifying the type of AE from among a huge variety. Both saturated fatty acids such as lauric acid or palmitic acid and unsaturated fatty acids such as oleic acid are used for the synthesis of AE, and compari-

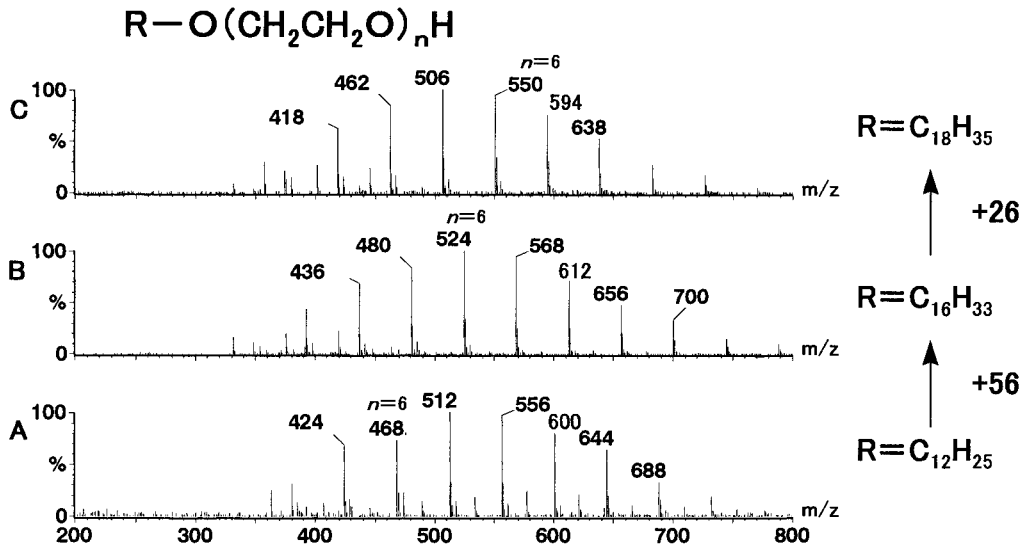

Fig. 4. Mass Spectra of Various Types of Polyoxyethylene Alkyl Ether
A, $C_{12=0}$–AE; B, $C_{16=0}$–AE; C, $C_{18=1}$–AE.

son of the difference in the mass units between known and unknown AE samples can result in the identification of the alkyl group of AE.

Sample Preparation

Surfactants have often been extracted by ODS-cartridge,[3,4] carbon cartridge,[5] XAD-16,[6] or liquid-liquid extraction.[4] We examined the sample preparation of low-concentration surfactant aqueous solution using 1) extraction with the weak anion-exchange cartridge Bond Elut® PSA; 2) extraction with the weak cation-exchange cartridge Bond Elut® CBA; 3) liquid-liquid extraction with tert-buthylmethylether; and 4) concentration in a water bath. As samples, we selected anionic surfactants LAS and AES, cationic surfactant BZ, and nonionic surfactant AE in aqueous solutions of 20 ppm. The recovery of surfactant was described as the mean recovery of three components selected from the total contents. The following procedures were performed:

1) The Bond Elut® PSA cartridge was activated by washing successively with 3 ml of methanol, 3 ml of pure water, and 3 ml of 28% ammonia-methanol (2 : 98, v/v). The aqueous sample (20 ppm, 20 ml) was applied to the cartridge, and the cartridge was subsequently washed with 1 ml of pure water and 1 ml of methanol. Retained surfactants were eluted with 1 ml of 28% ammonia-methanol (2 : 98, v/v). The eluate was evaporated to dryness under a gentle stream of nitrogen.

2) The Bond Elut® CBA cartridge was activated by washing successively with 3 ml of methanol, 3 ml of pure water, and 3 ml of 0.001 M HCl-methanol (2 : 98, v/v). The aqueous sample (20 ppm, 20 ml) was applied to the cartridge, and the cartridge was subsequently washed with 1 ml of pure water and 1 ml of methanol. Retained surfactants were eluted with 1 ml of 0.001 M HCl-methanol (2 : 98, v/v). The eluate was evaporated to dryness under a gentle stream of nitrogen.

3) Aqueous sample (20 ppm, 20 ml) and tert-buthylmethylether (20 ml) were added to a glass centrifuge tube and vortex mixed for 5 min, followed by centrifugation at 3000 rpm for 10 min. The organic layer was transferred to a glass container, and the aqueous layer was re extracted with an additional 20 ml of tert-buthylmethylether in the same manner.

4) The aqueous sample (20 ppm, 20 ml) was transferred to a glass evaporating dish and concentrated to dryness in a water bath.

The residues obtained from the four procedures were finally reconstituted in 0.2 ml of 50% methanol, with 5-μl aliquots submitted to LC/MS analysis.

When the ion-exchange cartridges were used, anionic surfactants (LAS and AES) and cationic surfactant (BZ) were successfully extracted with anion-exchange and cation-exchange cartridges, respectively. Their recovery rates were 53.8–107%, as summarized in Table 1. Nonionic surfactant (AE) was expected to pass through both anionic- and cationic-exchange cartridges, and therefore we analyzed

Table 1. Extraction Recovery Rates of Surfactants from Spiked Water Samples with Various Pretreatment Methods

	Recovery (%, mean ± S.D., $n = 3$)			
	Cation-exchange cartridge	Anion-exchange cartridge	Liquid-liquid extraction	Concentrated to dryness
LAS	22.9 ± 2.7	76.7 ± 8.2	33.5 ± 4.7	112 ± 7.0
AES	20.8 ± 2.9	53.8 ± 1.2	15.7 ± 2.5	124 ± 12
AE	3.23 ± 0.0	2.21 ± 0.0	38.3 ± 0.9	65.8 ± 3.6
BZ	107 ± 17	5.33 ± 0.1	29.0 ± 2.2	95.0 ± 7.6

the solutions that passed through the cartridge after applying sample. The recovery rates with cation-exchange and anion-exchange cartridges were only 3.23% and 2.21% (Table 1), and no components of AE were detected from either the washing solution or the eluates. These results suggest that most AE was absorbed on the extraction cartridges and did not elute from them. On the other hand, all surfactants were extracted by liquid-liquid extraction. However, the recovery rates were not satisfactory, ranging from 15.7–38.3%. When aqueous solutions of these surfactants were concentrated to dryness, the recovery rates ranged from 65.8% to 124%. These results indicate that concentration to dryness is the preferable preparation method for samples including aqueous solution, tea, *etc.*, which would contain less interfering substances. For the extraction of anionic and cationic surfactants, the use of ion-exchange cartridges was more effective than liquid-liquid extraction. In addition, the cation-exchange cartridge has been estimated to be more suitable for BZ than other solid-phase extraction cartridges such as the polymeric cartridge.[9]

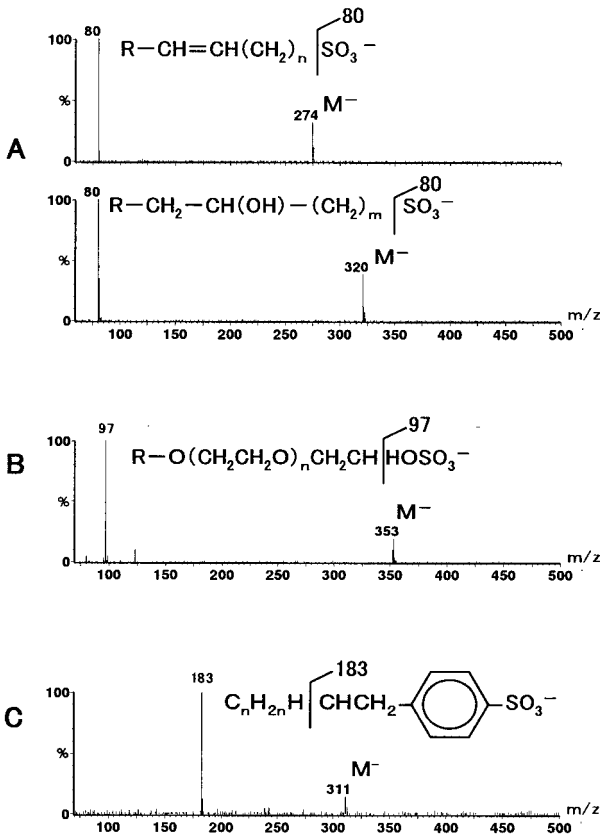

Fig. 5. Product Ion Spectra of Alpha-Olefine Sulfate (A), Polyoxyethylene Alkyl Ether Sulfate (B), and Linear Alkylbenzenesulfonate (C)

The collision energy was set at 20 V (for A) or 30 V (for B and C).

Structural Identification Using LC/MS/MS

The possibility of structural identification of surfactants from spectral information using LC/ESI-/MS/MS was examined. As samples, we selected anionic surfactants and attempted to identify the structure of their hydrophilic moieties by measuring the product ion spectra of AOS, AES, and LAS in the product ion-scan mode, in which each M^- ion was employed as a precursor ion.

AOS has two types of structure (Fig. 1), and they produced similar product ion spectra, represented by a predominant ion at *m/z* 80 originating from $-(SO_3)-$ (Fig. 5A). The predominant ions at *m/z* 97 originating from $-(SO_3OH)-$ and at *m/z* 183 from $-(CHCH_2C_6H_6SO_3)-$ were observed in the product ion spectra of AES and LAS, respectively (Figs. 5B and 5C). Based on these results, the structures of hydrophilic moieties in surfactants can be identified using LC/ESI-MS/MS.

Application to Criminal Investigations

Case 1: It was suspected that a victim himself had added detergent to a carton of orange juice. We analyzed the juice sample and detergents found in the suspect's home. The orange juice sample was diluted 5-fold with pure water, filtered through a 45-μm membrane filter, and subjected to LC/ESI-MS in the negative mode. The confiscated detergent

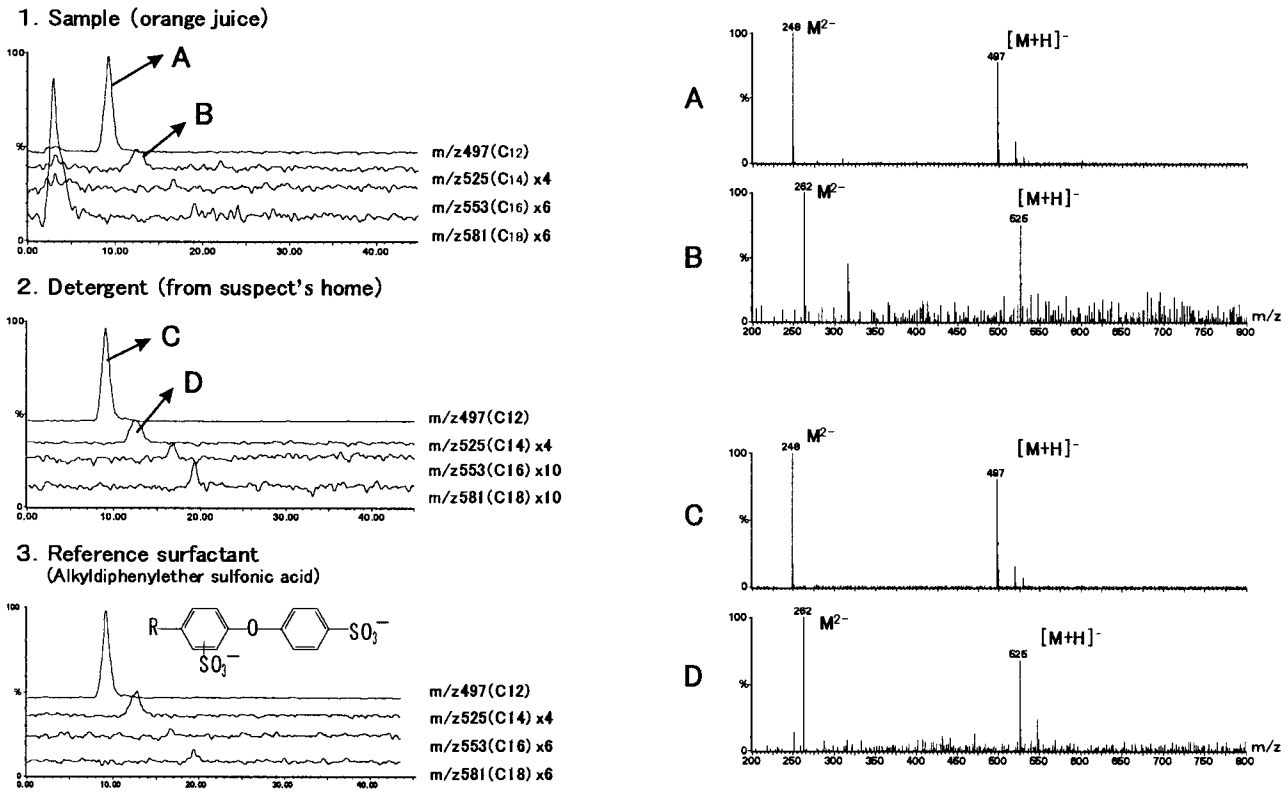

Fig. 6. Extracted Ion Chromatograms Obtained for Orange Juice (1), Detergent from a Suspect's Home (2), and Standard Alkyldiphenylether Sulfonic Acid (3) Using LC/ESI-MS

Instrument, Micromass Platform (ESI); column, L-column ODS; mobile phase, gradient of eluent A [methanol-ammonium acetate 10 mM (50 : 50, v/v)] and eluent B [methanol-ammonium acetate 10 mM (95 : 5, v/v)] (the concentration of eluent B was linearly increased from 40% to 100% in 20 min, and then maintained for 30 min); flow rate, 0.1 ml/min.

samples were diluted 20-fold with pure water and then prepared in the same manner.

Figures 6-1 and 6-2 show the mass chromatograms obtained from the orange juice and the detergent samples. Similar patterns of mass chromatograms and mass spectra were obtained from both the juice and the detergent samples. The standard surfactant (alkyldiphenylether sulfonic acid) was further analyzed under the same conditions (Fig. 6-3). The ingredients in the standard surfactant have two $-(SO_3^-)-$ moieties in each molecule, and a doubly charged molecular ion (M^{2-}) as a base peak and less intense $[M+H]^-$ ion in their mass spectra were observed. The mass chromatogram and the mass spectra patterns were identical with those from the surfactants in both the orange juice and the detergent. From the results obtained, we were able to identify the surfactant and confirmed that the suspect added put his own detergent to the orange juice.

Case 2: Detergent was found in bottled mineral water. We analyzed the sample using LC/ESI-MS in negative and positive modes. As shown in Fig. 7A, peaks appeared on the chromatogram in the negative mode, and a specific mass spectrum was obtained by integrating approximately from 18 to 22 min (Fig. 7B). These ingredients were identified as polyoxyethylene dodecyl ether sulfate (C_{12}–AES). However, in the positive mode, a peak was obtained at approximately 24.5 min (Fig. 7C). The mass spectrum for this peak is shown in Fig. 7D. Careful examination of the mass spectrum revealed that the components were polyoxyethylene dodecyl ether (C_{12}–AE).

Case 3: A canned coffee drink was subjected to LC/ESI-MS in the negative and the positive modes. The coffee sample was diluted 20-fold with pure water and filtered through a 45-μm membrane filter. Although the pretreatment was extremely simple and easy, no interference was observed on the mass chromatograms and mass spectra. After careful examination of the mass spectra, the surfactants in the coffee sample were identified as C_{10}–C_{13}–LAS (Figs. 8A and 8B) and C_{12}–AO (Figs. 8C and 8D) with the spectrum characterized by relatively intense

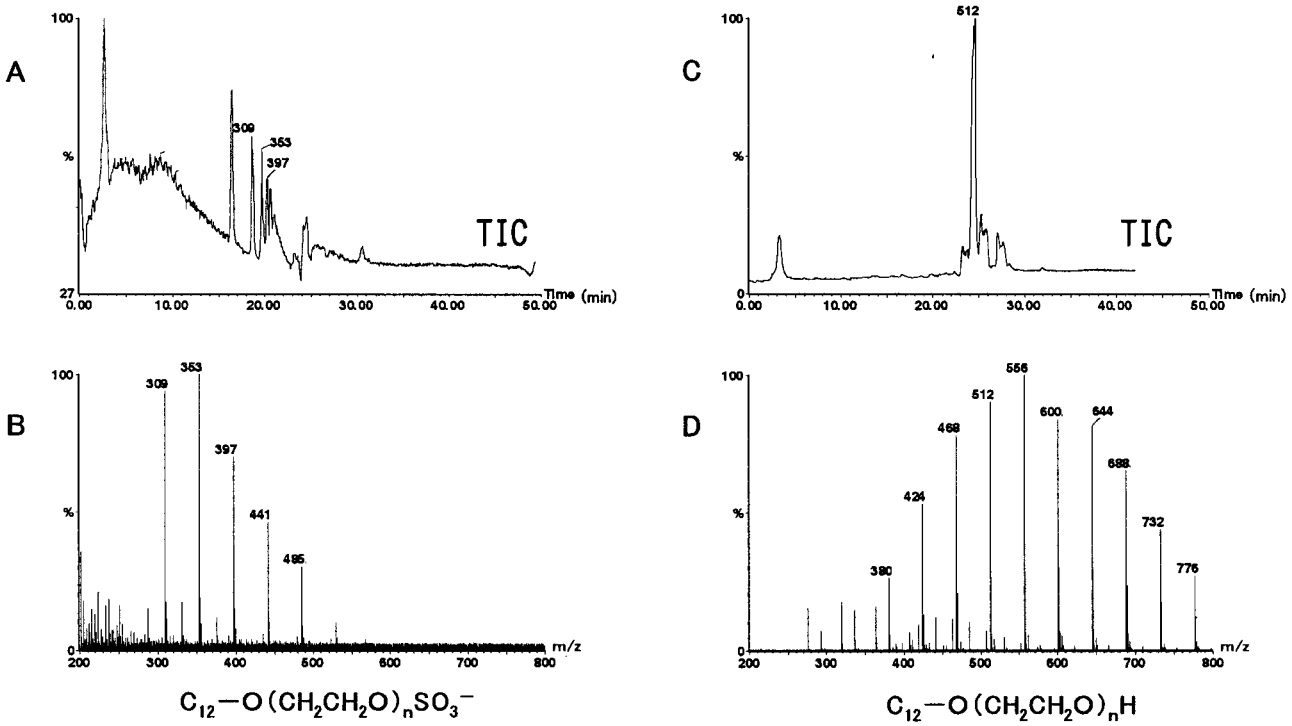

Fig. 7. Total Ion Chromatogram (TIC) in Negative Mode (A), Mass Spectra of Polyoxyethylene Dodecyl Ether Sulfate (B), TIC in Positive Mode (C), and Mass Spectrum of Polyoxyethylene Dodecyl Ether (D), Obtained from a Mineral Water Sample to Which Detergent was Added

The conditions for LC/ESI-MS were the same as in Fig. 6.

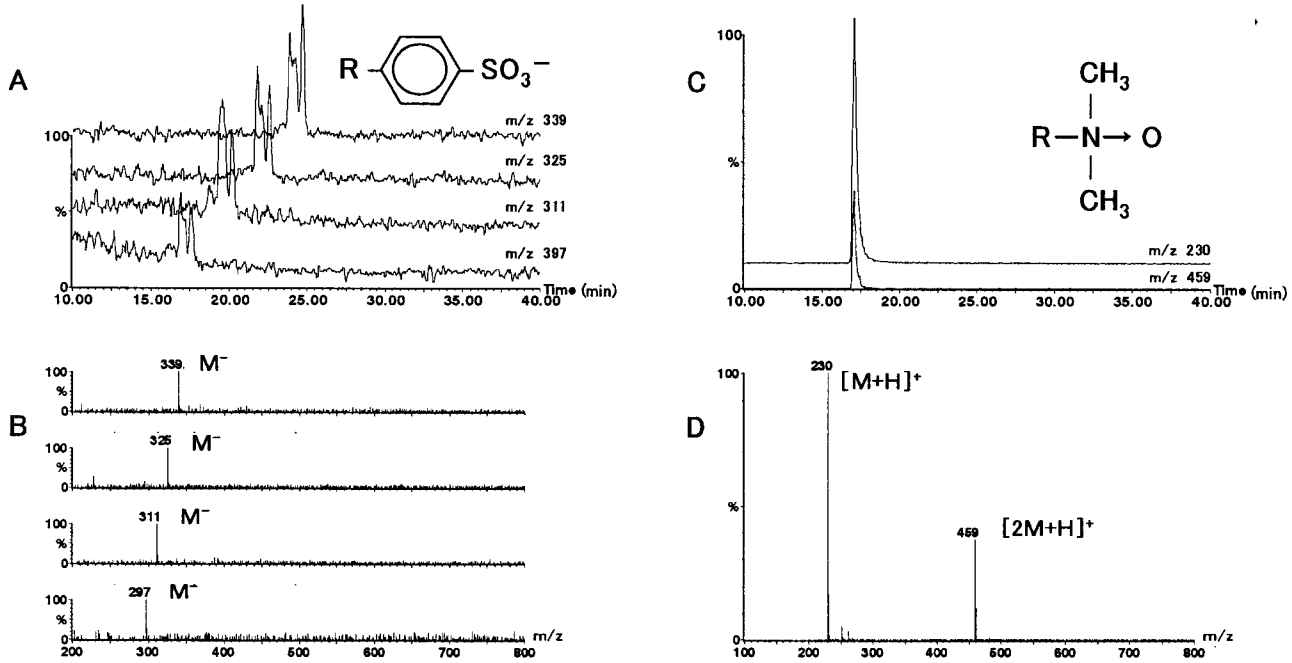

Fig. 8. Total Ion Chromatogram in Negative Mode (A), Mass Spectra of Linear Alkylbenzene Sulfonate (B), Total Ion Chromatogram in Positive Mode (C), and Mass Spectrum of Alkyl Dimethyl Amine Oxide (D) Obtained from a Canned Coffee Sample to Which Detergent was Added

The conditions for LC/ESI-MS were the same as in Fig. 6.

$[2M+H]^+$ ions along with more intense $[M+H]^+$ ions.

Neither extraction nor concentration of surfactants from the three samples was required, because the concentration of mixed surfactants in the samples was high. However, extraction and concentration processes would be required for samples containing lower concentration of surfactants.

In conclusions, anionic, cationic, and nonionic surfactants were analyzed in both the negative and positive mode. For anionic and cationic surfactants, M^- ions in the negative mode and M^+ ions in the positive mode were observed, respectively. On the contrary, nonionic surfactants yielded $[M+H]^+$ ions or $[M+NH_4]^+$ ions in the positive mode. Using the MS technique developed after sample preparation by simply diluting with pure water and filtering through a 45-μm membrane filter, detergent mixed in beverages was successfully identified. The method described in this paper will therefor be a powerful method for the identification and discrimination of surfactants in forensic aqueous samples.

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