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Determination of linear alkylbenzene sulfonates in water samples by liquid chromatography–UV detection and confirmation by liquid chromatography–mass spectrometry

Sunanta Wangkarn *, Phimpha Soisungnoen, Mongkon Rayanakorn, Kate Grudpan

Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

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Abstract

A high-performance liquid chromatographic (HPLC) method was developed for the separation and determination of individual (C_{10}–C_{13}) linear alkylbenzene sulfonates (LAS). New sets of conditions have been established for routine analysis of individual chemical forms of four LAS surfactants, i.e. C_{10}–C_{13} LAS. Under a condition set using a mobile phase containing 1.5 mM ammonium acetate in methanol/water 80:20 (v/v) mixture, detection limits obtained were in the range 1.5 ppb (for C_{10} LAS) to 11.5 ppb (for C_{13} LAS). This offers the advantages of significant improvement in resolution, short separation time and using less amount of common salt under isocratic condition. In addition, the use of simple mobile phase containing a simple low amount of salt cannot deposit at the entrance of mass spectrometric detector. The method is applicable to the simultaneous determination of LAS surfactants in various water samples. LAS surfactants presented in these samples were also successfully confirmed by using electrospray mass spectrometry.

Keywords: Anionic surfactant; Linear alkylbenzene sulfonates; HPLC; Mass spectrometry; Water

1. Introduction

Linear alkylbenzene sulfonates (LAS), synthetic anionic surfactants, have been used in household laundry and dish-washing detergents [1]. The commercial product is a mixture of homologues containing carbon atoms between 10 and 13 atoms. Each of these homologues consists of positional isomers resulting from the attachment of the phenyl ring to the carbon atoms of the linear alkyl chain (Fig. 1) [2,3]. They are rapidly biodegraded under aerobic conditions. LAS-containing detergents are used in large quantities, and are therefore, released in the environment. LAS homologues with alkyl chain lengths from C_{10} to C_{13} have been found in municipal wastewaters and sediments at the ppm levels [4–6]. It has been reported that LAS and their degradation products can affect membrane permeability, enzyme and lysosomal activity [7,8]. The toxicity of the LAS containing 13 carbon atoms to the microalgae, namely *Chaetoceros gracitis* was found to be greater than that of the C_{11} LAS [9]. For these reasons, the identification and quantification of individual LAS species are invaluable for estimating the environmental impact and potential health effects of LAS species.

The standard methylene blue method has long been used for determining total amounts of sulfonate- and sulfate-based anionic surfactants in wastewater. Although the method cannot differentiate individual anionic surfactant, it is normally expressed as methylene blue-active substances (MBAS) concentrations down to 25 ppb [10]. It is time consuming and is often interfered by sample matrix, i.e. organic sulfonates, sulfates, carboxylates and phenol. This method also requires a large quantity of the toxic solvent for extraction, such as chloroform.

A number of methods have been developed for identifying and quantifying individual chemical forms of anionic surfactants. Chromatographic techniques like gas chromatography

* Corresponding author. Tel.: +66 53 943341; fax: +66 53 892277.
E-mail address: sunanta@chiangmai.ac.th (S. Wangkarn).

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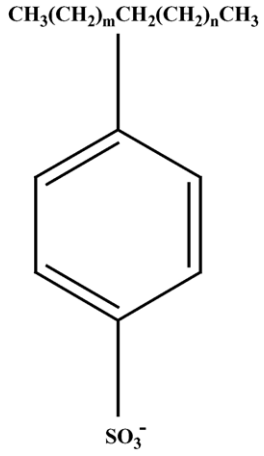

Fig. 1. General chemical structure of LAS.

(GC) [4,11–15], electrophoresis [1] and high-performance liquid chromatography (HPLC) [16–18] are efficient separation methods for the analysis of LAS mixture. Due to their low volatility and anionic form, derivatization of these compounds is necessary, when GC-based method is used [19].

HPLC is currently a suitable method for the determination of LAS. Reversed-phase HPLC provides a good separation of LAS mixture when using various chromatographic detectors, e.g. ultraviolet (UV) [16–18], fluorescence [2,20,21] and mass spectrometry (MS) [22–24]. However, most existing methods and procedures are still far from being considered suitable for the routine determination of individual chemical forms of LAS. Most HPLC methods with UV detector require the mobile phase containing either sodium perchlorate [22] or additive mixture, such as triethylamine and acetic acid [23,24], trifluoroacetic acid (TFA) and tetrabutylammonium dihydrogenphosphate (TBA–H_2PO_4) [25] or cetyltrimethylammonium ($CTMA^+$) ions [26] in order to resolve LAS homologues under gradient conditions. In practical application of the mobile phase containing high amount of those compounds, particularly sodium perchlorate ($10\,g\,l^{-1}$) can shorten the column life and can also clog the capillary, when mass spectrometric detector is used. In addition, the complicated mass spectra of LAS homologues would be obtained. This makes the identification of individual LAS in environmental samples so difficult.

As reported earlier by other workers, LAS compounds containing 10–13 carbon atoms are used in large amounts and are, therefore, released in the environment [1,4–6]. The toxicity of surfactant to aquatic organisms increases with increasing of carbon atoms [9].

The main purpose of this study was to develop HPLC method that would allow for routine analysis of LAS mixture, particularly in respect to reducing analysis time, improving separation efficiency for all the four LAS surfactants, precision and accuracy under isocratic condition. As mentioned earlier, several publications have been reported to use complicated mixtures as mobile phase under gradient conditions for separating some LAS surfactants. These approaches can also cause either capillary blockage or additional spectral interferences, when a mass spectrometric detector is used for confirmation results. Therefore, common salts, i.e. sodium chloride, sodium acetate and ammonium acetate added into mobile phase were chosen because of their suitability of identifying LAS in water samples using mass spectrometric detection.

2. Experimental

2.1. Chemicals

Linear alkylbenzene sulfonates in the forms of sodium salts were obtained from Henkel (Germany). HPLC-grade methanol was purchased from BDH (Poole, England). Sodium chloride, sodium acetate and ammonium acetate were analytical grade and purchased from Carlo Erba (Barcelona, Spain). Milli-Q water was used in this study.

2.2. Instrumentation

A HP 1100 high-performance liquid chromatograph (Agilent Corp., Wilmington, USA) consisting of an Agilent 1100 quaternary pump and an Agilent 1100 UV detector (224 nm) was employed. An inlet frit with 2 μm pore size was placed between the injector and HPLC column. A Zorbax Eclipse XDB C_8 column (Agilent Corp., USA), 15 cm × 4.6 mm i.d., containing 5 μm diameter packing material was used.

Samples were injected onto this column via an injection valve filled with 20 μl loop. The mobile-phase system was the mixture of methanol/water containing various amounts of sodium chloride, sodium acetate or ammonium acetate at flow rate $1.0\,ml\,min^{-1}$. All chromatographic elutions were isocratic and carried out at room temperature.

A HP 1100 series mass-selective detector single quadrupole instrument equipped with the orthogonal spray–ESI (Agilent, USA) interface was used for these investigations. The fragmentor voltage, nebulizer pressure, drying gas flow rate, drying gas temperature and capillary voltage were set to 150 V, 20 psi, $10\,l\,min^{-1}$, 350 °C and 3500 V, respectively.

2.3. Sample preparation

Prior to HPLC analysis, water samples were subjected to purification and preconcentration on the Sep-Pak C_{18} cartridge (Waters, USA). The cartridge was preconditioned with 7 ml methanol, followed by 7 ml of deionised water and then the sample was passed through the cartridge. The cartridge was washed with 6 ml of the mixture of MeOH–H_2O (30:70, v/v) and was eluted with 3 ml of methanol.

3. Results and discussion

The optimization of LAS separation using reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection at 224 nm was achieved, employing Eclipse XDB C_8 column with 15 cm × 4.6 mm dimension and 5 μm diameter packing material. Optimum separation of LAS homologues containing 10–13 carbon atoms was obtained by appropriately adjusting the composition of mobile phase, type and the concentration of salt. The emphasis was placed on the use of common salts (sodium chloride, sodium acetate and ammonium acetate), instead of using sodium perchlorate, in order to avoid capillary blockage and high background signal when using mass spectrometric detection.

3.1. Effect of mobile-phase composition

Mobile-phase compositions in the range of 70–80% (v/v) methanol in water were investigated. Separation of a mixture of C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS was carried out as depicted in Fig. 2. LAS compounds were separated using 70% methanol in water as mobile phase. However, most peaks obtained were broad, particularly C_{13} LAS. It was also observed that the LAS compounds containing 10 and 11 carbon atoms were not resolved completely when using 75% methanol. No separation was observed when using the amounts of methanol exceeding 80%. It is evident from these chromatograms that the composition of mobile phase affects peak resolution and peak shape significantly. It was noticed that peak resolution deteriorated with increasing methanol content. This could be explained that surfactants

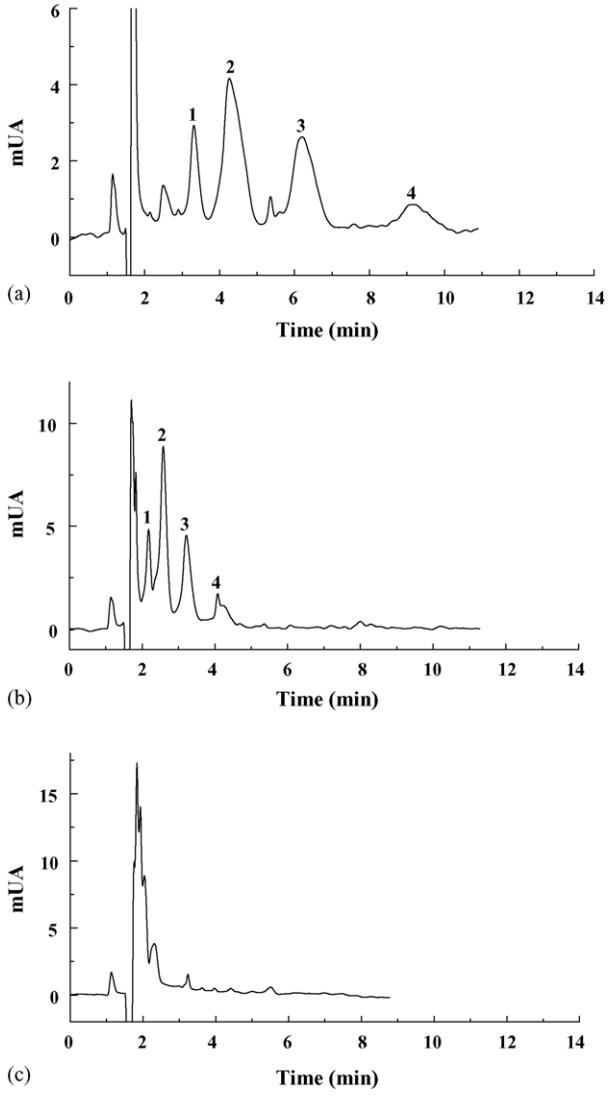

Fig. 2. Chromatograms of mixture of four LAS compounds obtained using various mobile-phase compositions of MeOH–H_2O: (a) 70:30; (b) 75:25; (c) 80:20. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

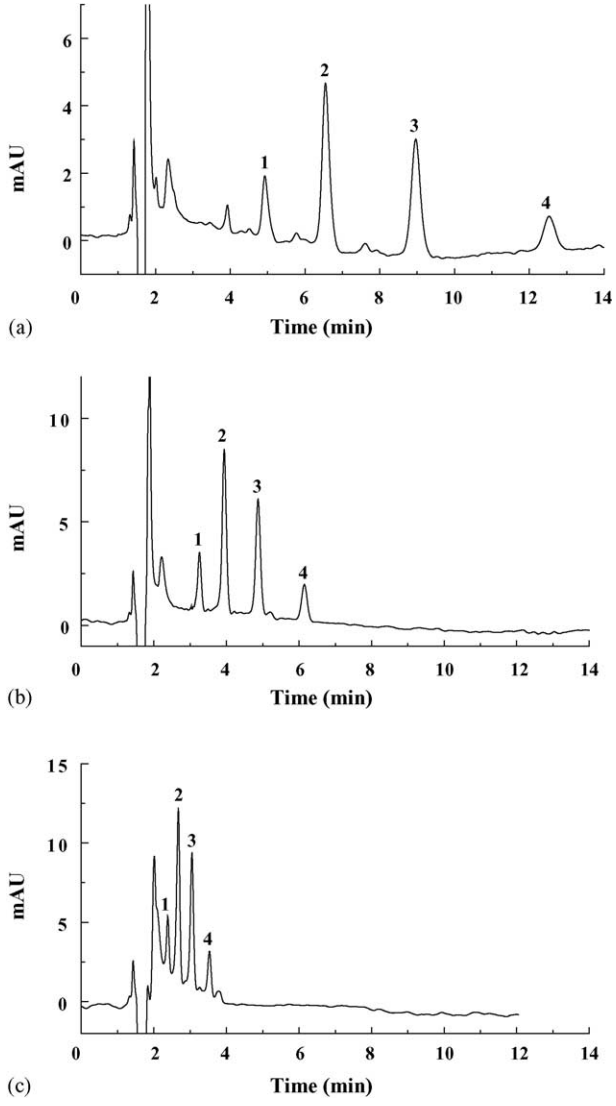

Fig. 3. Chromatograms of mixture of four LAS compounds obtained using various mobile-phase compositions in the presence of 3.5 mM NaCl and MeOH–H_2O: (a) 75:25; (b) 80:20; (c) 85:15. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

are hydrophobic in nature. The hydrophobic characteristic of long-chain surfactants is suppressed by increasing methanol resulting in reducing retention time [27].

3.2. Effect of type and concentration of salt

Preliminary experiments were undertaken in an attempt to find suitable salt adding into mobile phase for improving LAS separation. Sodium chloride was common salt used for separations of LAS mixture as shown in Fig. 3. Four LAS compounds were successfully resolved within 6 min when using the 80:20 (v/v) mixture of methanol and water containing 3.5 mM NaCl. When using the 75:25 (v/v) mixture of methanol and water containing 3.5 mM NaCl the same four LAS compounds were separated in over 12 min. It was also observed that C_{10} LAS and C_{11} LAS were partially resolved when using the amounts of methanol exceeding 85%. The mixture of methanol/water (80:20, v/v) was, therefore, selected for further method development.

As demonstrated earlier, the selection of a suitable common salt is a critical factor in obtaining optimum resolution and short separation times. Three types of common salts, i.e. sodium chloride, sodium acetate and ammonium acetate were investigated at the concentrations ranging from 1 to 10 mM adding into the mixture of methanol/water (80:20, v/v) along with a mobile-phase flow rate 1.0 ml min^{-1}. It was observed that the resolution and their retention time increased with the concentration of salt (Figs. 4–6). The minimum concentrations of sodium chloride, sodium acetate and ammonium acetate that could be used to separate the four LAS compounds (resolution ≥ 1.5) in approximately 5 min under isocratic condition were 1, 2 and 1.5 mM, respectively. In comparing the data obtained in this study to the data reported elsewhere [28] indicated that the developed method provided a significantly improved resolution, peak shape, particularly C_{12} LAS, short analysis time and simple approach for confirmation results by mass spectrometric detector.

3.3. Linearity, accuracy and detection limit

Linearity, accuracy and detection limit for individual LAS compounds were studied (Table 1). The mobile phase

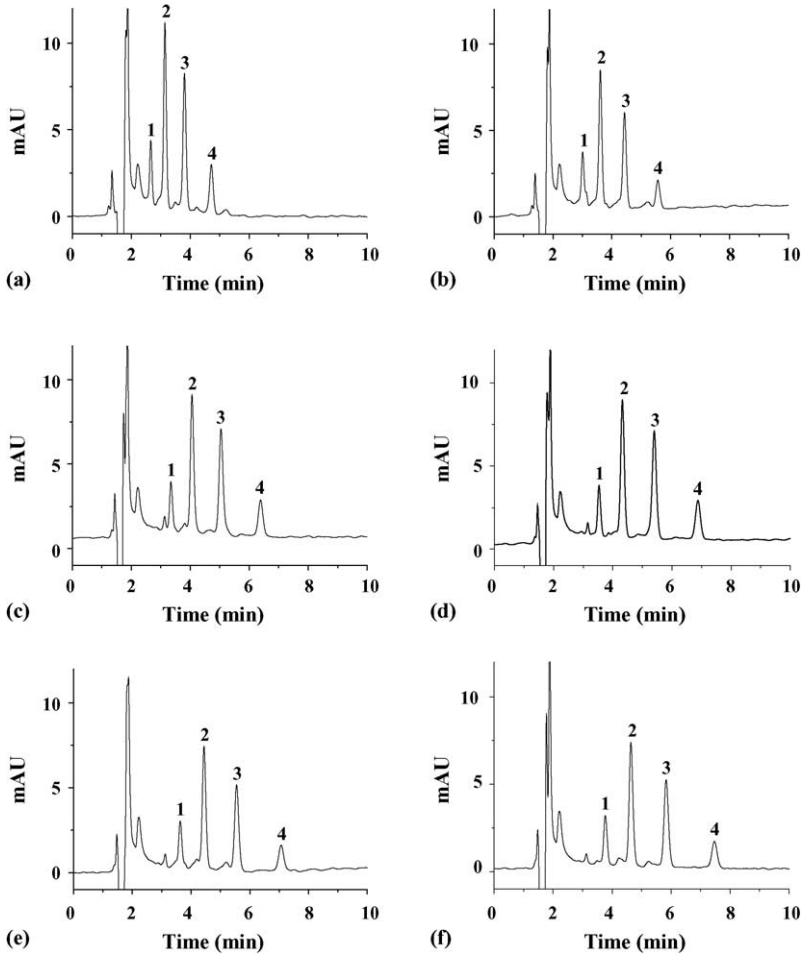

Fig. 4. Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium chloride: (a) 1 mM; (b) 2 mM; (c) 4 mM; (d) 6 mM; (e) 8 mM; (f) 10 mM. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

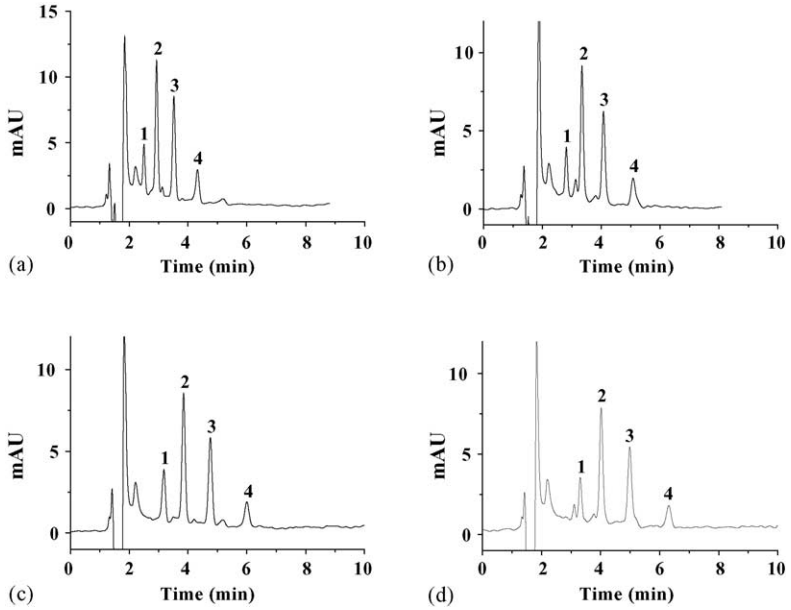

Fig. 5. Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium acetate: (a) 2 mM; (b) 4 mM; (c) 8 mM; (d) 10 mM. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

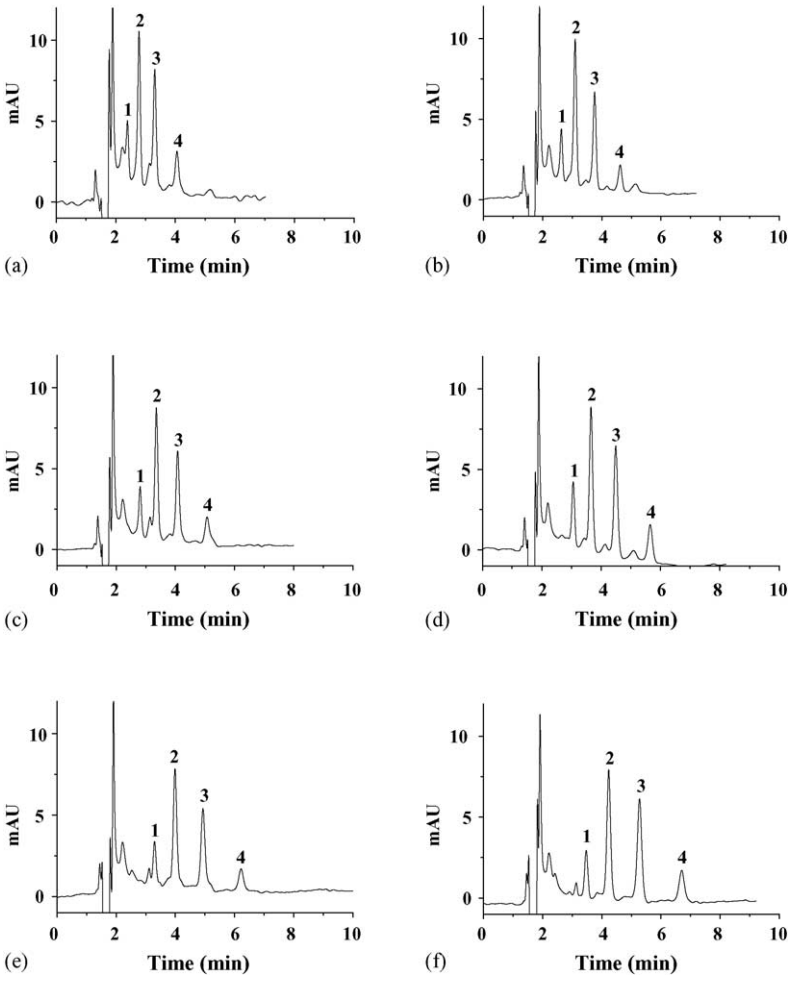

Fig. 6. Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of ammonium acetate: (a) 1 mM; (b) 1.5 mM; (c) 2 mM; (d) 4 mM; (e) 6 mM; (f) 8 mM. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

Table 1
Analytical merits of the proposed method (linearity, accuracy and detection limit)

Compound	Concentration range (ppb)	R^2	Linearity cut-off (ppb) × 10^3	%Recovery	Detection limit[a] (ppb)
C_{10} LAS	6–310	0.9984	41	91–101	1.5
C_{11} LAS	20–1030	0.9997	137	92–99	8.0
C_{12} LAS	20–1070	0.9994	143	95–99	7.0
C_{13} LAS	20–590	0.9979	78	94–102	11.5

[a] Calculation based on three times the background standard deviation.

containing 80% methanol and 1.5 mM ammonium acetate in water were selected for this study due to the results obtained previously in compromising between resolution ($R_s \geq 1.5$) and analysis time (within 5 min). The accuracy expressed in terms of percentage recovery was done by spiking various amounts of LAS standard into the water samples collected from wastewater in Chiang Mai and Utraradit, Thailand. The percentage recoveries of this method for C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS were found to be between 91 and 101 ($n = 3$), 92 and 99 ($n = 3$), 95 and 99 ($n = 3$) and 94 and 102 ($n = 3$), respectively. Satisfactory recovery was obtained. The limit of detection for each LAS compound was calculated as three times the background standard deviation [29]. The C_{10} LAS had the lowest detection limit of 1.5 ppb. The C_{13} LAS, which is the last compound to elute under the conditions employed, had the highest detection limit value of 11.5 ppb. From the observation, the peak shape of C_{13} LAS is rather broader than that of C_{10} LAS.

3.4. Analysis of LAS surfactant in real water samples using HPLC–UV

Prior to real sample analysis, results obtained with the four LAS standards indicated that an increase of methanol from 75 to 80% is expected to cause C_{10} LAS and C_{11} LAS to elute close to some probable interferences (Fig. 6b). The mobile phase containing 1.5 mM ammonium acetate in methanol/water mixture of 78:22 (v/v), instead of the ratio of 80:20 (v/v) methanol/water, was used in order to avoid matrix effects arising from the water extracts. These effects cause approximately 2 min increase in the separation time of LAS compounds. As for water extracts, the chromatograms in Fig. 7 illustrate the separation of the four compounds for various water samples. Under the condition, the LAS compound concentrations in various water samples determined using a Zorbax Eclipse XDB C_8 column in combination with the methanol/water mixture containing ammonium acetate, are presented in Table 2.

3.5. Identification of LAS surfactants in water extract using LC–ES–MS

It is well known that the identification of LAS compounds using chromatographic techniques is based solely on retention-time matching. As a consequence, errors can result from using this approach, especially in the case of co-eluting compounds. Also of particular interest is the numerous unknown anionic surfactants that have been found in environmental samples when analysing them using HPLC–UV [17–19].

To overcome such problems, the negative-ion electrospray (ES)–mass spectrometry was used for confirmation of LAS compounds in water samples. The mass spectra of water extracts (Fig. 8) show the *m/z* 183 ion common to LAS compounds. In addition, the high intensity of the molecular ions observed at *m/z* 297, 311, 325 and 339 originating from the water extracts are similar to those originating from the LAS

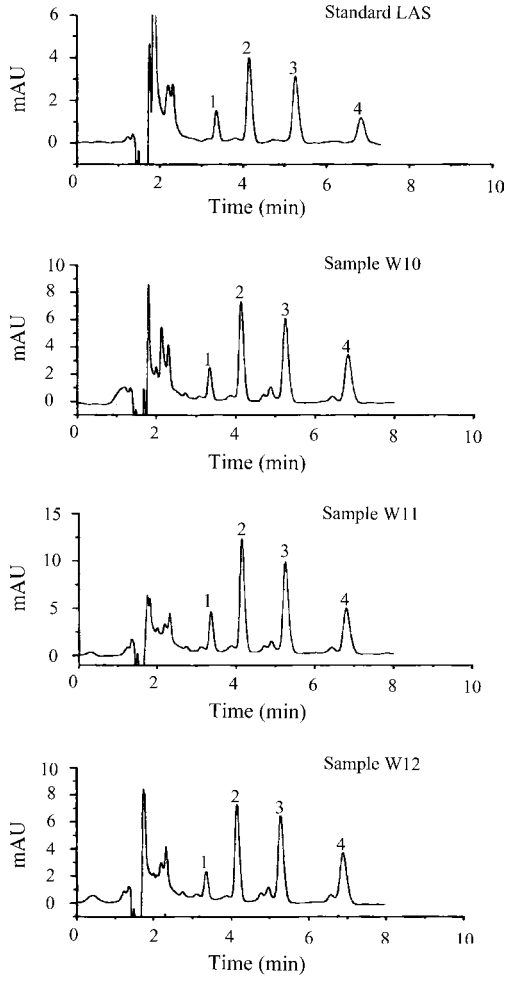

Fig. 7. Chromatograms of mixture of four LAS compounds in standard solution and water extracts obtained using 78% (v/v) methanol in water containing 1.5 mM ammonium acetate. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

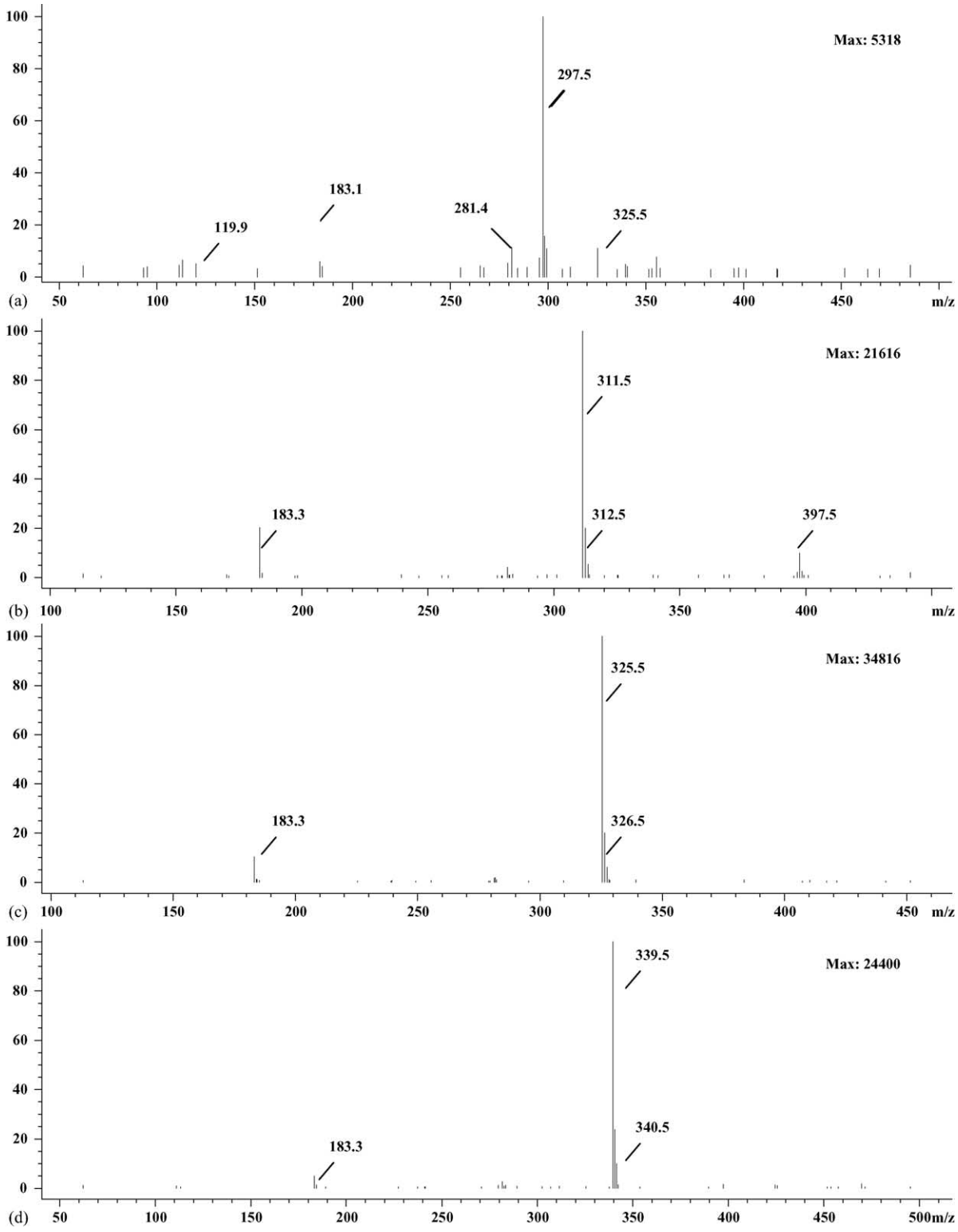

Fig. 8. (a–d) Negative-ion ESI mass spectra of LAS compounds originating from water extract (W10).

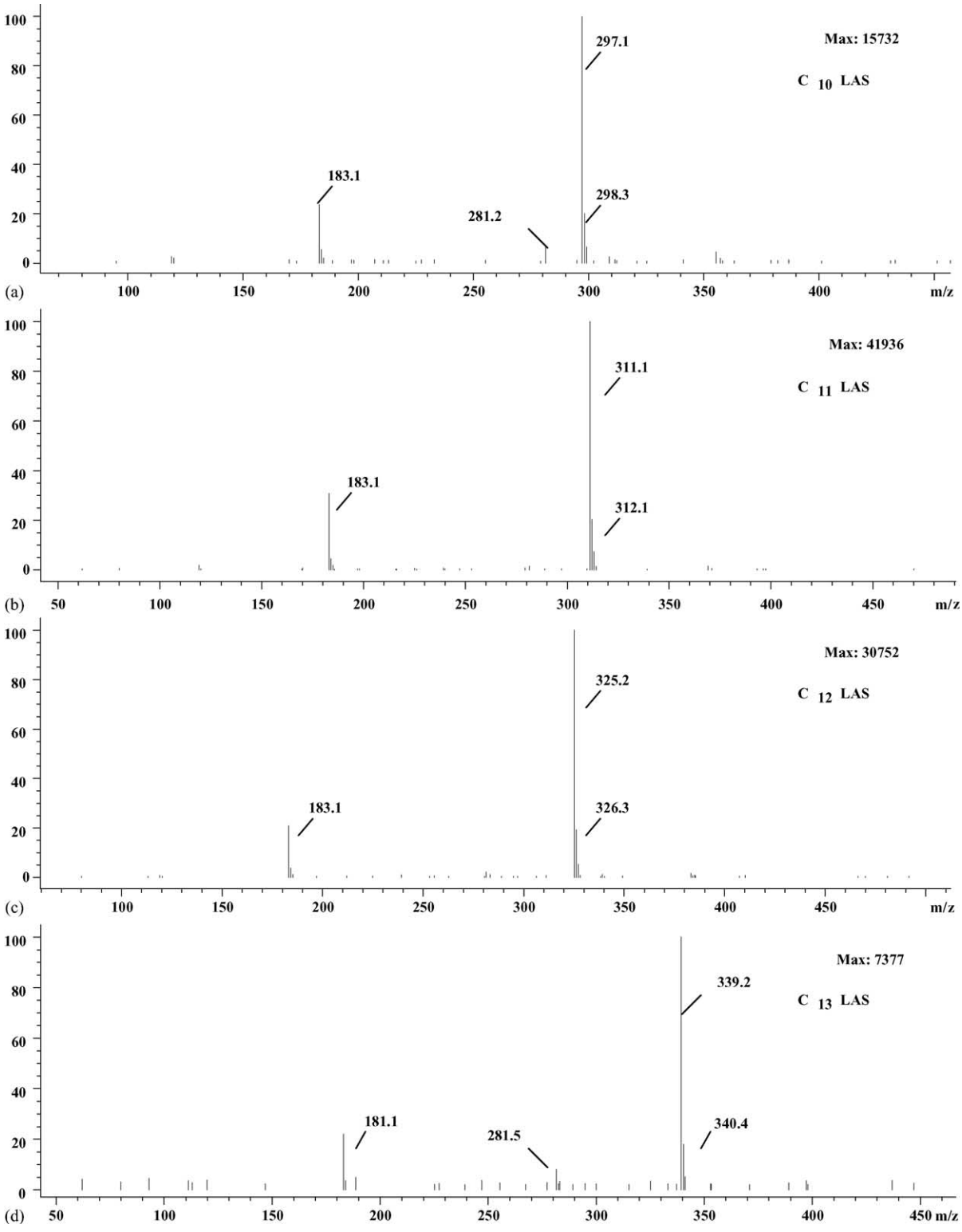

Fig. 9. (a–d) Negative-ion ESI mass spectra of the molecular ion originating from the mixture of LAS standard.

Table 2
Determination of LAS compounds in water samples (mean ± S.D.; $n = 3$)

Type of water	Concentration (ppb)			
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS
W1	115.3 ± 2.7	303.2 ± 7.9	184.4 ± 8.0	81.9 ± 2.2
W2	310.0 ± 0.3	1173.4 ± 4.6	1145.0 ± 12.9	424.4 ± 5.0
W3	5.0 ± 0.1[a]	23.4 ± 0.5	26.5 ± 1.1	21.1 ± 1.5
W4	n.d.	n.d.	13.0 ± 0.9	14.8 ± 0.5
W5	n.d.	n.d.	n.d.	n.d.
W6	3.4 ± 0.1[a]	12.8 ± 0.9	15.1 ± 1.3	16.7 ± 1.6
W7	54.1 ± 1.9	120.1 ± 5.5	69.1 ± 4.8	36.6 ± 5.2
W8	n.d.	n.d.	n.d.	n.d.
W9	n.d.	n.d.	n.d.	n.d.
W10	8.8 ± 0.3[a]	30.9 ± 0.7	33.5 ± 1.7	25.2 ± 1.7
W11	14.5 ± 0.8[a]	48.0 ± 2.4	46.9 ± 3.5	28.9 ± 2.8
W12	6.5 ± 0.4[a]	27.8 ± 1.9	30.3 ± 2.1	22.6 ± 1.3

n.d., not detected (less than the detection limit value); W1, drainage water from student dormitory, Chiang Mai University; W2, wastewater from Center of Medical Sciences, Ministry of Public Health; W3, wastewater from Khuy Hospital, Utraradit Province; W4, domestic wastewater released into Thorn canal, Utraradit Province; W5, natural water in Mae-Ping River, Chiang Mai Province; W7, wastewater in Mae-Kha canal, Chiang Mai Province; W8, natural water in Ang-Kaew reservoir, Chiang Mai University; W9, water in Chiang Mai Moat, Chiang Mai Province; W6 and W10–W13, natural water from irrigation canal, Chiang Mai Province.

[a] Preconcentration as described in Section 2.3.

standards (Fig. 9). These ions correspond to C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS, respectively.

4. Conclusion

The developed method offers superior performance characteristics, i.e. a simple method, significant improvement in resolution, short analysis time and using less amount of common salt (1.5 mM ammonium acetate) under isocratic condition. With this regard, it is easy to use this method with a mass spectrometric detector without any blockage of MS capillary. In addition, the use of low amounts of salt also increases the column's life and only requires very short re-equilibration time between each injection. Overall, these features demonstrate that the method is suitable to be used for routine analysis for both the identification and quantification of individuals of C_{10}–C_{13} LAS surfactants in various water samples.

Acknowledgements

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