CHAPTER 3

RESULTS AND DISCUSSION

In this work, some anionic surfactants (LAS and SDS) and nonionic surfactant (Triton X-100) were analyzed using RP-HPLC. The Zorbax Eclipse XDB C_8 column was used for the optimization of HPLC conditions and analysis of these compounds in water samples. The experimental study was divided into three parts, i.e. LAS separation, LAS and Triton X-100 analysis and SDS analysis.

3.1 Separation of LAS Compounds

3.1.1 Optimization of HPLC conditions

The optimum HPLC conditions for the separation of LAS homologues containing 10 to 13 carbon atoms was achieved by adjusting the composition of mobile phase, type and concentration of common salts, i.e. sodium chloride, sodium acetate and ammonium acetate.

3.1.1.1 Detection wavelength

The 5 ppm LAS standard solution was scanned the absorption in the wavelength between 200 to 350 nm using UV-VIS spectrophotometer. It was found that the maximum absorption wavelength (λ_{max}) of LAS standard was found to be about 224 nm as shown in **Fig. 3.1** which closely to detection wavelength reported elsewhere [37, 39].

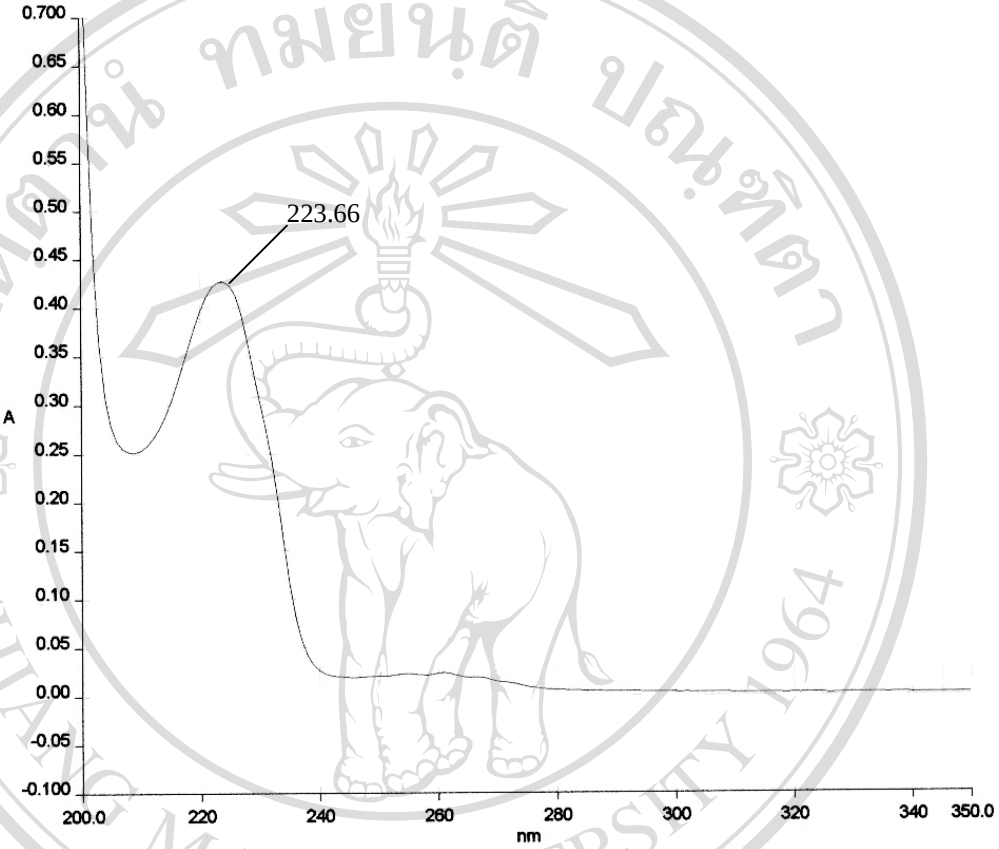

Fig. 3.1 Absorption spectrum of 5 ppm LAS standard solution.

3.1.1.2 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. 3.2**. 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 are hydrophobic in nature. The hydrophobic characteristic of long-chain surfactants is suppressed by increasing methanol resulting in reducing retention time [44].

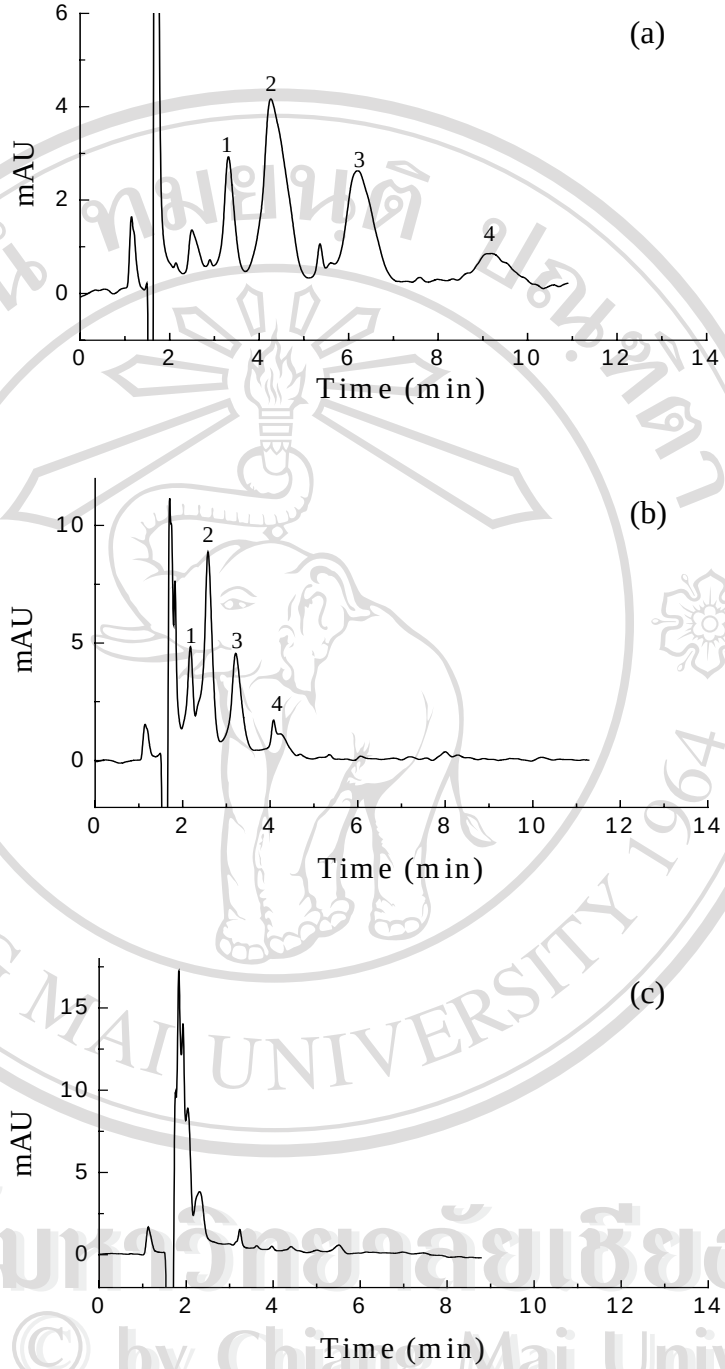

Fig. 3.2 Chromatograms of mixture of four LAS compounds obtained using various mobile phase compositions of MeOH-H_2O: (a) 70:30; (b) 75:25; (c) 80:20. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

3.1.1.3 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. 3.3**. 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 sodium chloride. When using the 75:25 (v/v) mixture of methanol and water containing 3.5 mM sodium chloride 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 reported by Chen and Pietrzyk [39], the addition of salt in mobile phase can improve peak shape and resolution of LAS. This could be explained that the increasing ionic strength of mobile phase affects to the retention of LAS on reversed stationary phase according to the electric double layer model. The LAS anions are retained on the stationary phase surface through a hydrophobic interaction between the alkyl chain of the anionic surfactants and the stationary phase. This creates a negatively charged primary layer at the stationary phase surface and a secondary diffuse layer of cations to maintain electrical neutrality. As mobile phase ionic strength increases, the interaction between the cation and the retained LAS increases. This reduces the anionic character of LAS surfactants, thus increasing the interaction between the LAS surfactants and the stationary phase.

It has been documented that 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. 3.4-3.6**). The minimum concentrations of sodium chloride, sodium acetate and ammonium acetate that could be used to separate the four LAS compounds ($R_S \geq 1.5$) in approximately 5 min under isocratic condition were 1, 2 and 1.5 mM, respectively [51]. In comparing the data obtained in this study to the data reported elsewhere [3] 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.

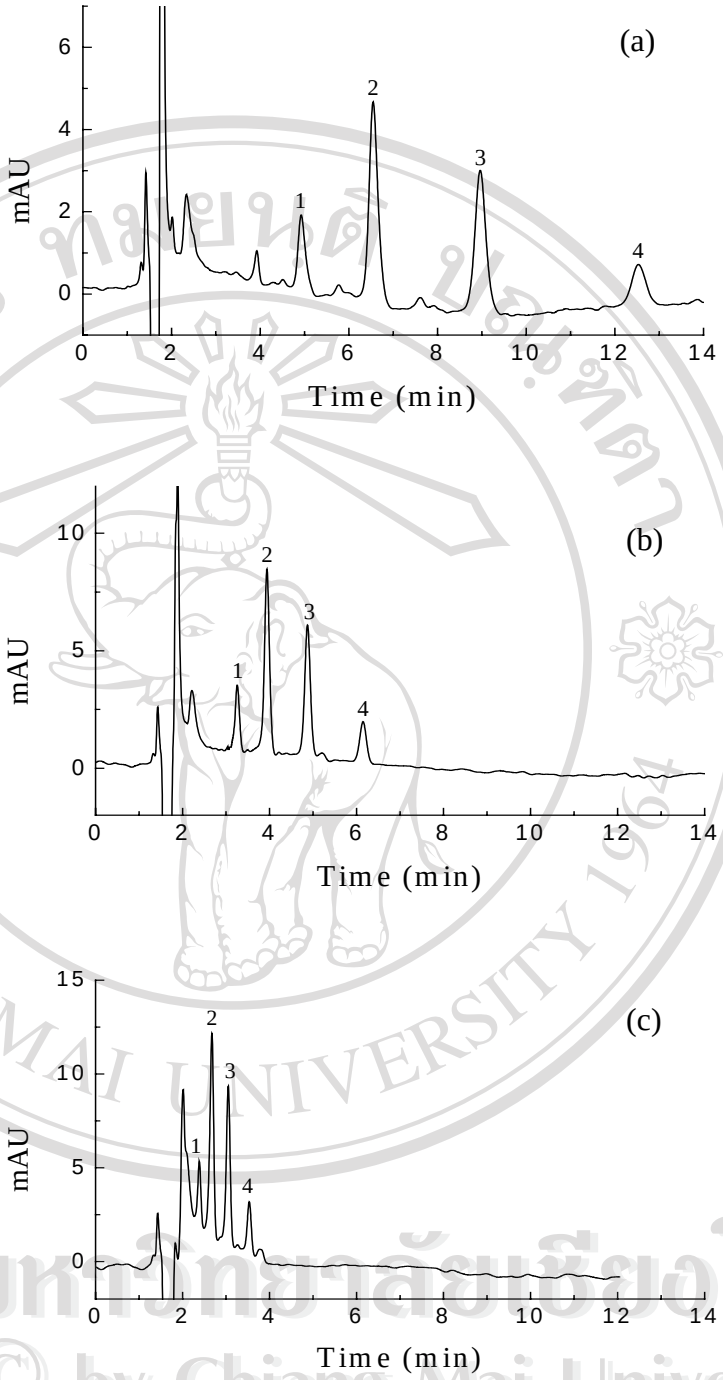

Fig. 3.3 Chromatograms of mixture of four LAS compounds obtained using various mobile phase compositions of MeOH-H_2O with the presence of 3.5 mM NaCl: (a) 75:25; (b) 80:20; (c) 85:15. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

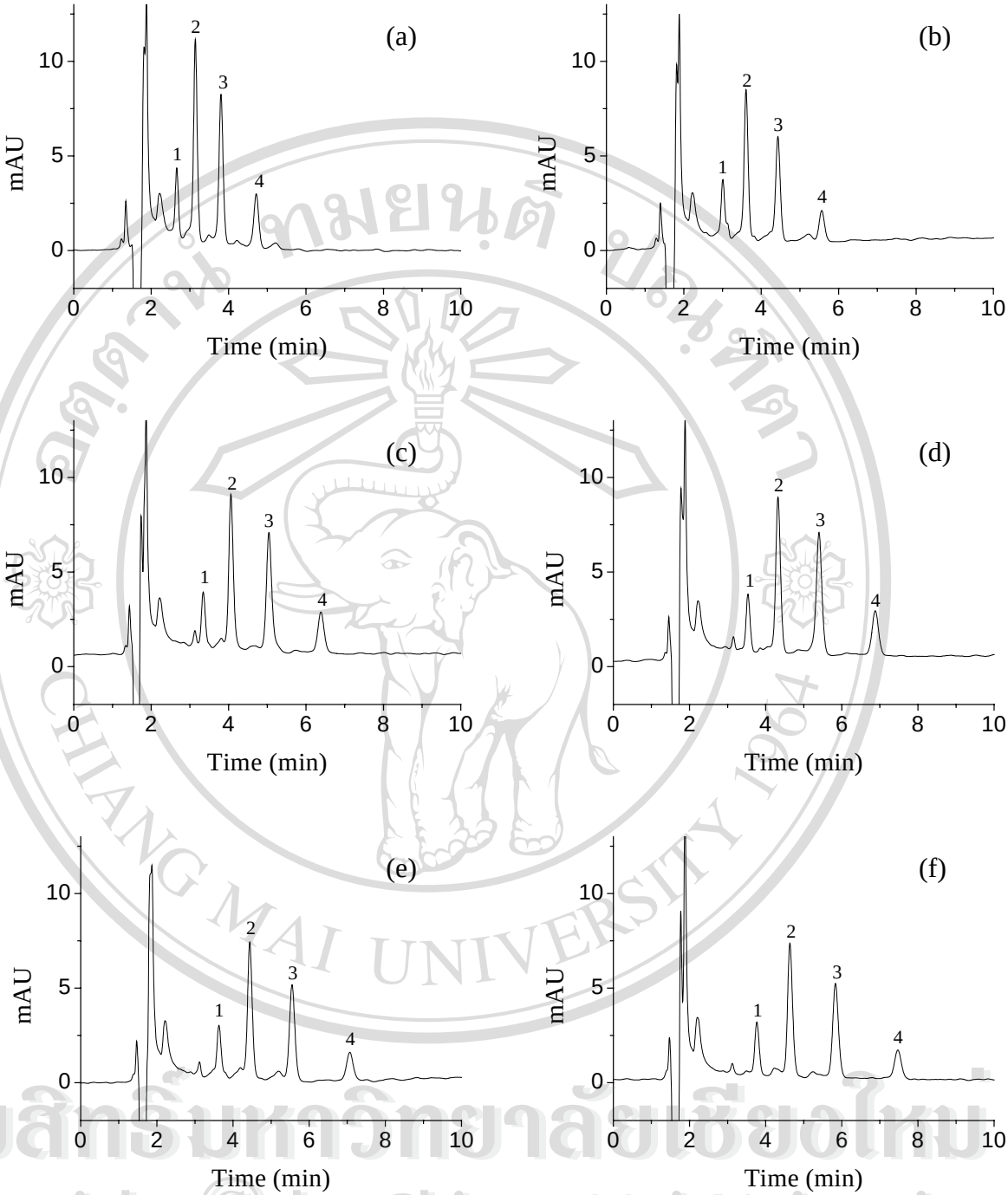

Fig. 3.4 Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium chloride: (a) 1 mM; (b) 2 mM; (c) 4 mM; (d) 6 mM; (e) 8 mM; (f) 10 mM.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

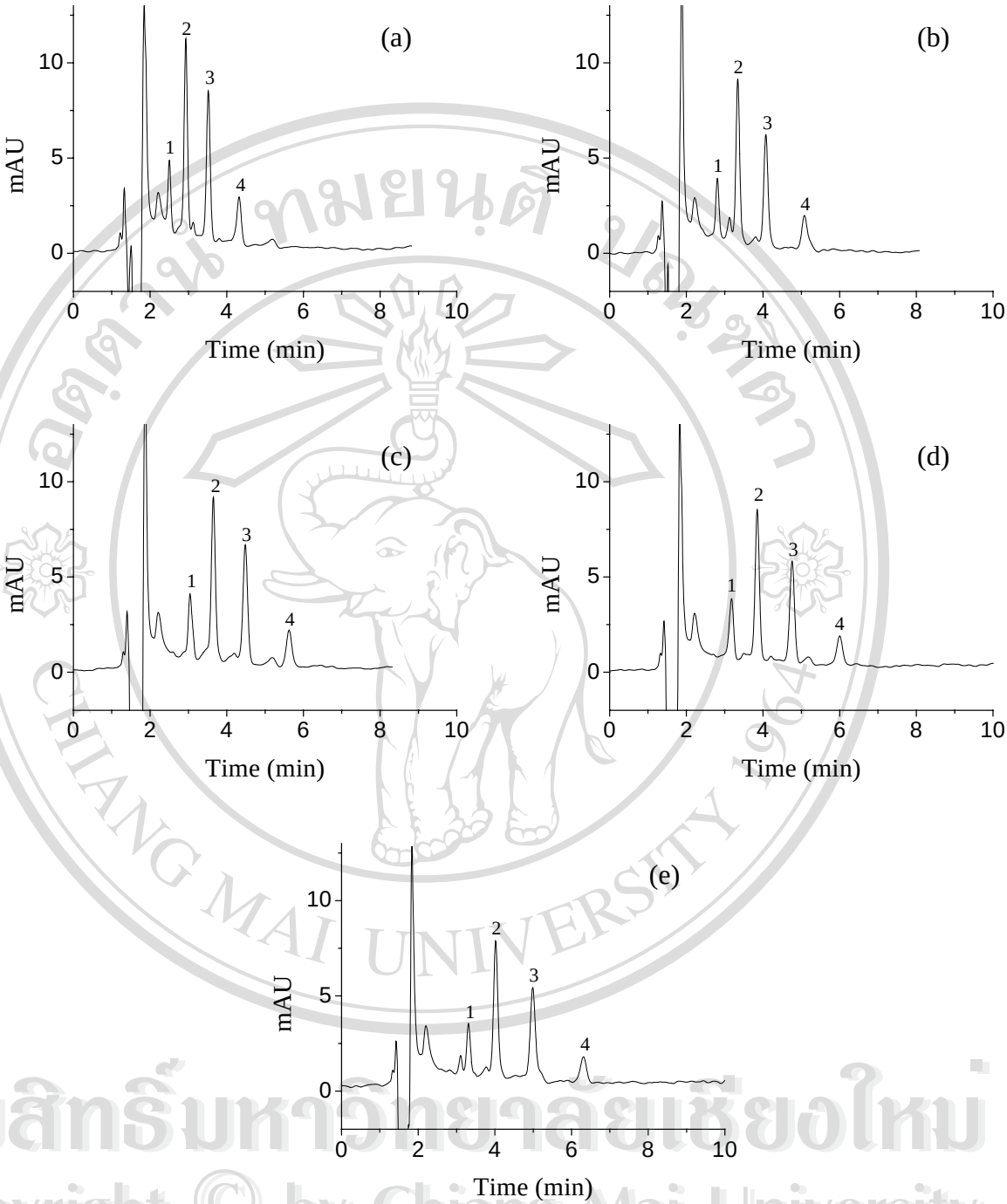

Fig. 3.5 Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of sodium acetate: (a) 2 mM; (b) 4 mM; (c) 6 mM; (d) 8 mM; (e) 10 mM.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

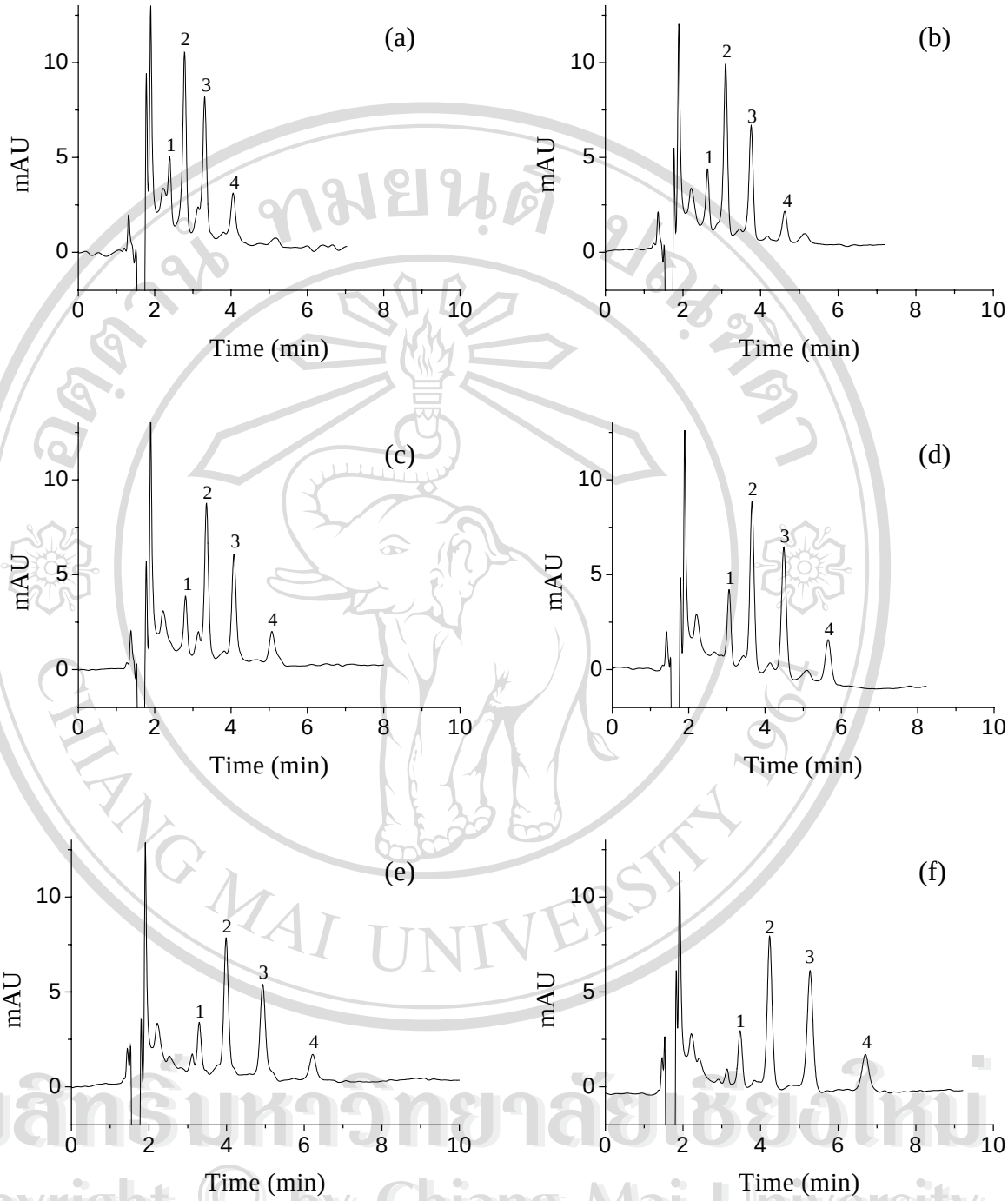

Fig. 3.6 Chromatograms of mixture of four LAS compounds obtained using 80% (v/v) methanol in water containing various concentrations of ammonium acetate: (a) 1 mM; (b) 1.5 mM; (c) 2 mM; (d) 4 mM; (e) 6 mM; (f) 8 mM.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

3.1.2 Confirmation of LAS homologues

As individual LAS compound is not available in the market, the negative-ion ES-MS was used in order to confirm identifications made by HPLC-UV. The mobile phase containing ammonium acetate was employed in order to avoid capillary blockage. Moreover, in ionization step ammonium acetate have been reported to evaporate easily when comparing with sodium chloride and sodium acetate [21]. The total ion chromatogram (TIC) and mass spectra of LAS compounds were depicted in **Figs. 3.7** and **3.8**, respectively. The ions occurring at *m/z* 297, 311, 325 and 339 corresponded to pseudomolecular ions of C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS, respectively, corresponding to $[M\text{-}Na]^-$. In addition, it was found the ion at *m/z* 183 (**Fig. 3.9**), corresponding to $[C_8H_7SO_3]^-$. This ion is known as the specific fragment ion for LAS compounds [45].

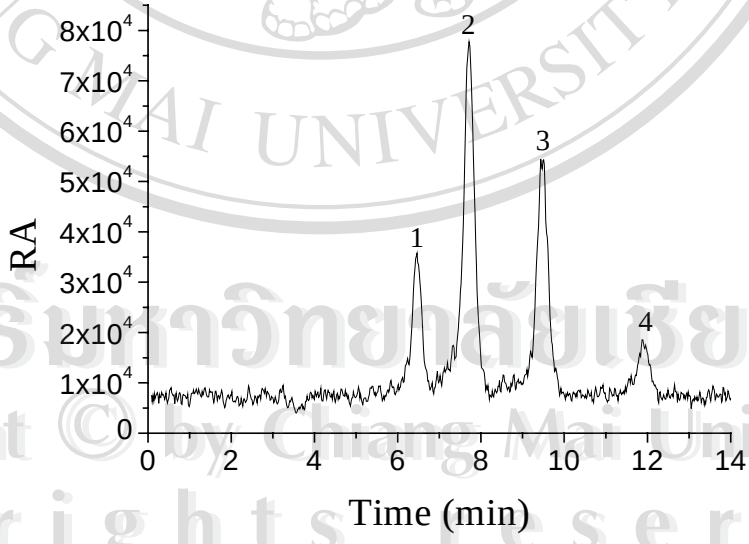

Fig. 3.7 Total ion chromatogram (TIC) of LAS compounds obtained using 80% (v/v) methanol in water containing 1.5 mM ammonium acetate.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

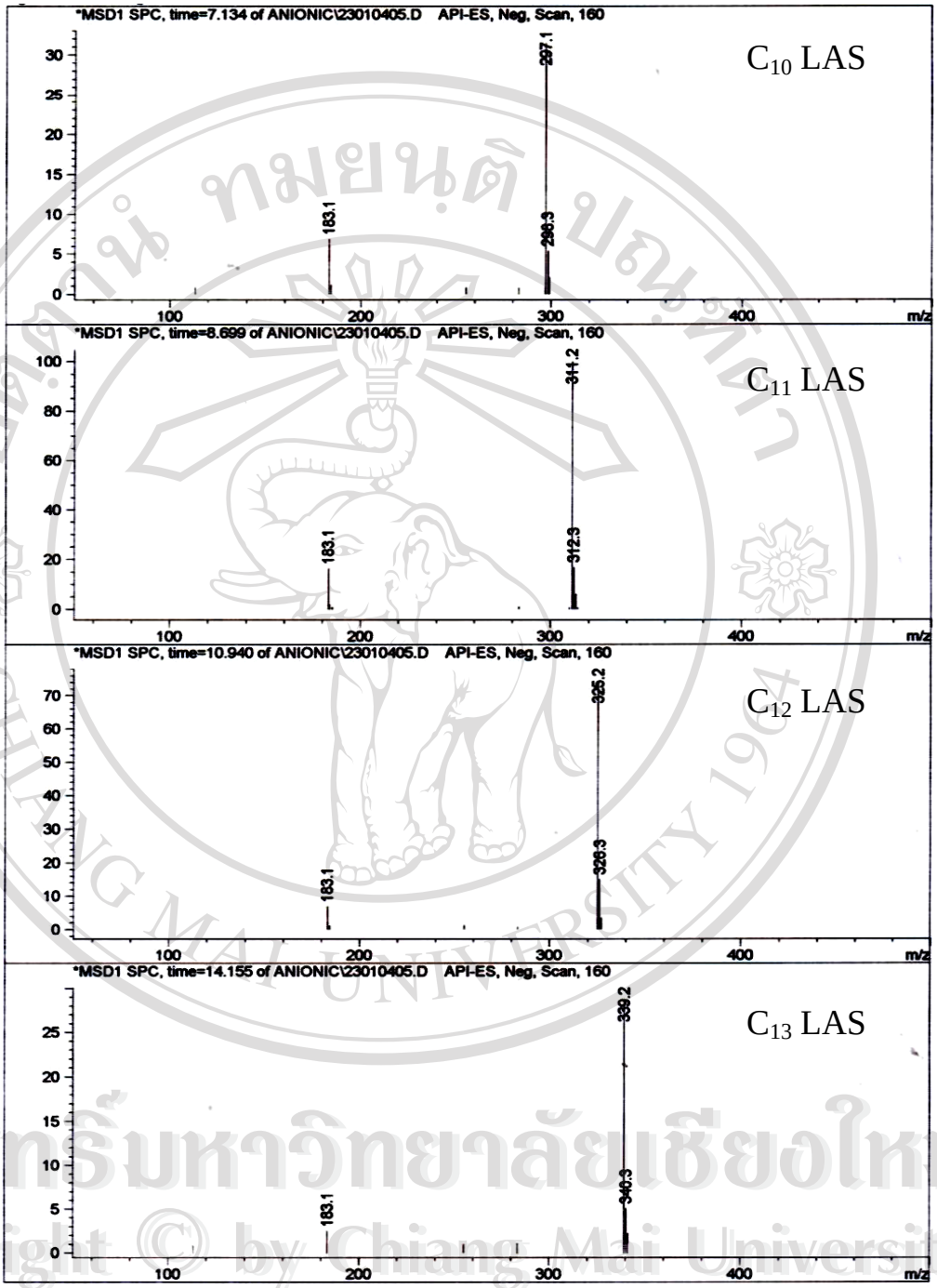

Fig. 3.8 Negative-ion ES mass spectra of LAS compounds originating from LAS standard.

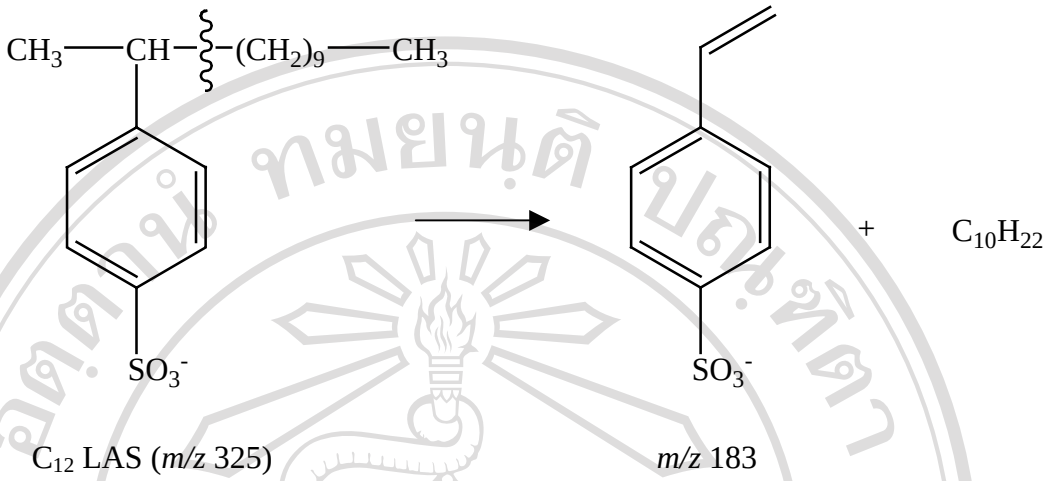

Fig. 3.9 Fragmentation of a C_{12} LAS isomer providing the specific fragment ion with *m/z* 183 [45, 52].

3.1.3 Composition of LAS homologues

As reported elsewhere [31] the composition of commercial LAS homologues purchased from different suppliers were different. For example, the approximate composition of LAS homologues obtained from Taiwan was C_{10} 13%, C_{11} 27%, C_{12} 48% and C_{13} 12% [31] whereas that obtained from Petroquímica Español (Spain) was C_{10} 3.9%, C_{11} 37.4%, C_{12} 35.4% and C_{13} 23.1% [42]. In this study, the composition of commercial LAS homologues was determined by injecting various concentrations of LAS compounds into the HPLC system. The percentage amounts of individual LAS compound were found to be C_{10} 10.4%, C_{11} 34.3%, C_{12} 35.8% and C_{13} 19.6% as shown in **Table 3.1**.

Table 3.1 Percentage amounts of commercial LAS homologues (n=3)

Concentration of LAS (ppm)	Amount (%)			
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS
1	10.2	33.1	36.2	20.5
2	10.3	34.6	35.2	19.9
3	10.6	34.6	35.9	19.0
4	10.4	34.9	35.8	18.9
average	10.4	34.3	35.8	19.6
% R.S.D.	1.3	2.4	1.2	3.7

3.2 Analysis of LAS and Triton X-100

3.2.1 Optimization of HPLC conditions

The investigated parameters included wavelength of detector as well as composition and flow rate of the mobile phase.

3.2.1.1 Detection wavelength

The 5 ppm LAS and 10 ppm Triton X-100 standard solutions were scanned the absorption in the wavelength range of 200-350 nm using UV-VIS spectrophotometer. It was observed that the maximum absorption wavelengths (λ_{max}) were found to be about 224 nm for both standard solutions as shown in **Fig. 3.10**. For the analysis of Triton X-100, Karlsson et.al. [47] reported that it could be detected at two wavelength ranges, i.e. 200-230 nm and 280 nm. As illustrated in **Fig. 3.10**, Triton X-100 gives a much higher absorbance at the wavelength of 223 nm compared to the response obtained at 280 nm. Consequently, the wavelength at 224 nm was suitable for the detection of LAS and Triton X-100.

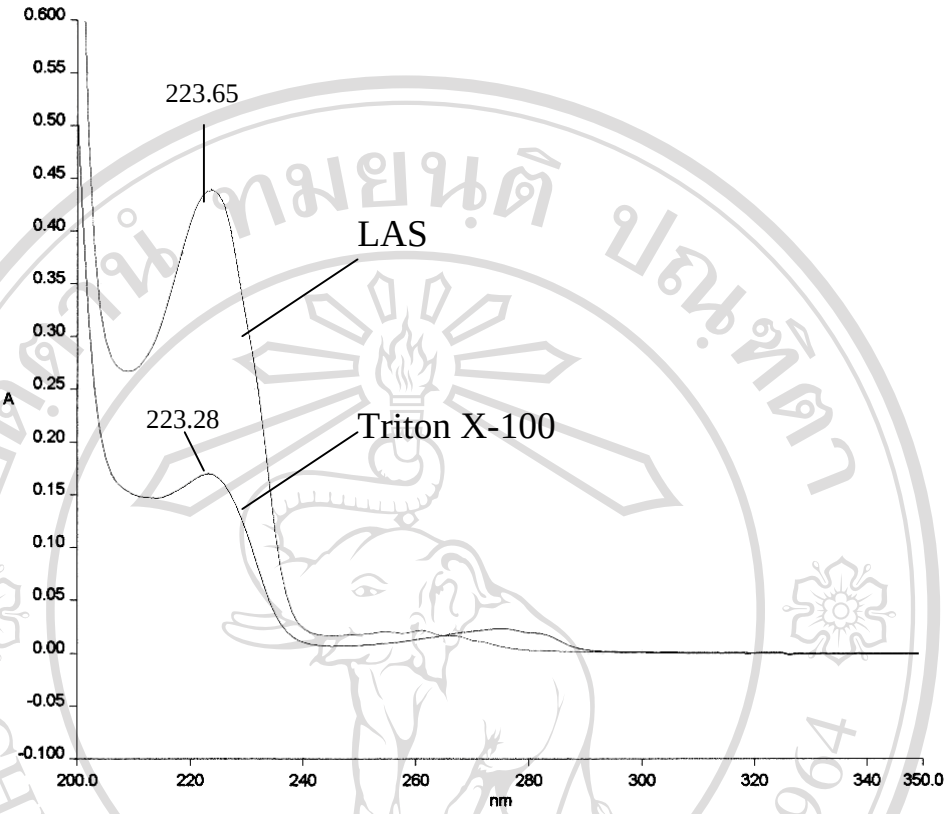

Fig. 3.10 Absorption spectra of LAS and Triton X-100 standard solutions.

3.2.1.2 Effect of mobile phase composition and concentration of ammonium acetate

The mobile phase containing ammonium acetate was employed in this study. The effect of concentrations of ammonium acetate in 75% and 80% (v/v) methanol in water was investigated in the range of 1-5 mM as depicted in **Figs. 3.11** and **3.12**. As shown in **Fig. 3.11**, the complete separation of five compounds (C_{10} LAS–C_{13} LAS and Triton X-100) was observed when using 75% methanol solution containing 2 or 3 mM ammonium acetate. The mobile phase containing 2 mM ammonium acetate provided the better resolution for all pairs, particularly between

C_{12} LAS and Triton X-100 (R_S > 1.5) within short analysis time (11 min). Peak overlap between C_{13} LAS and Triton X-100 was observed when using 1 mM ammonium acetate and peak overlap between C_{12} LAS and Triton X-100 was observed when using 4 mM ammonium acetate. When 80% methanol solution containing 3 mM ammonium acetate was used, the mixture of four LAS compounds and Triton X-100 were successfully resolved within 7 min (**Fig. 3.12**). It was also observed that C_{13} LAS and Triton X-100 was co-eluted when using 2 mM ammonium acetate. In addition, peak overlap between C_{12} LAS and Triton X-100 was obtained when using ammonium acetate exceeding 3 mM.

In order to achieve the LOD investigation further, the low concentration of standard mixture (0.2 ppm LAS and 0.5 Triton X-100) was injected into two HPLC systems, i.e. 80% methanol in water containing 3 mM ammonium acetate and 75% methanol in water containing 2 mM ammonium acetate as mobile phase. The resulting chromatograms were shown in **Fig. 3.13**. It was found that C_{11} LAS was overlapped with impurities when using 80 % methanol in water containing 3 mM ammonium acetate as depicted in **Fig. 3.13a**. The separation was achieved for all five compounds in approximately 12 min when using 75% methanol in water containing 2 mM ammonium acetate (**Fig. 3.13b**). Therefore, the optimum mobile phase for the separation of LAS compounds and Triton X-100 was the mixture of 75% methanol in water containing 2 mM ammonium acetate. When comparing with the reported elsewhere [53], it was found that the proposed method provided improved less amount of salt and short analysis time.

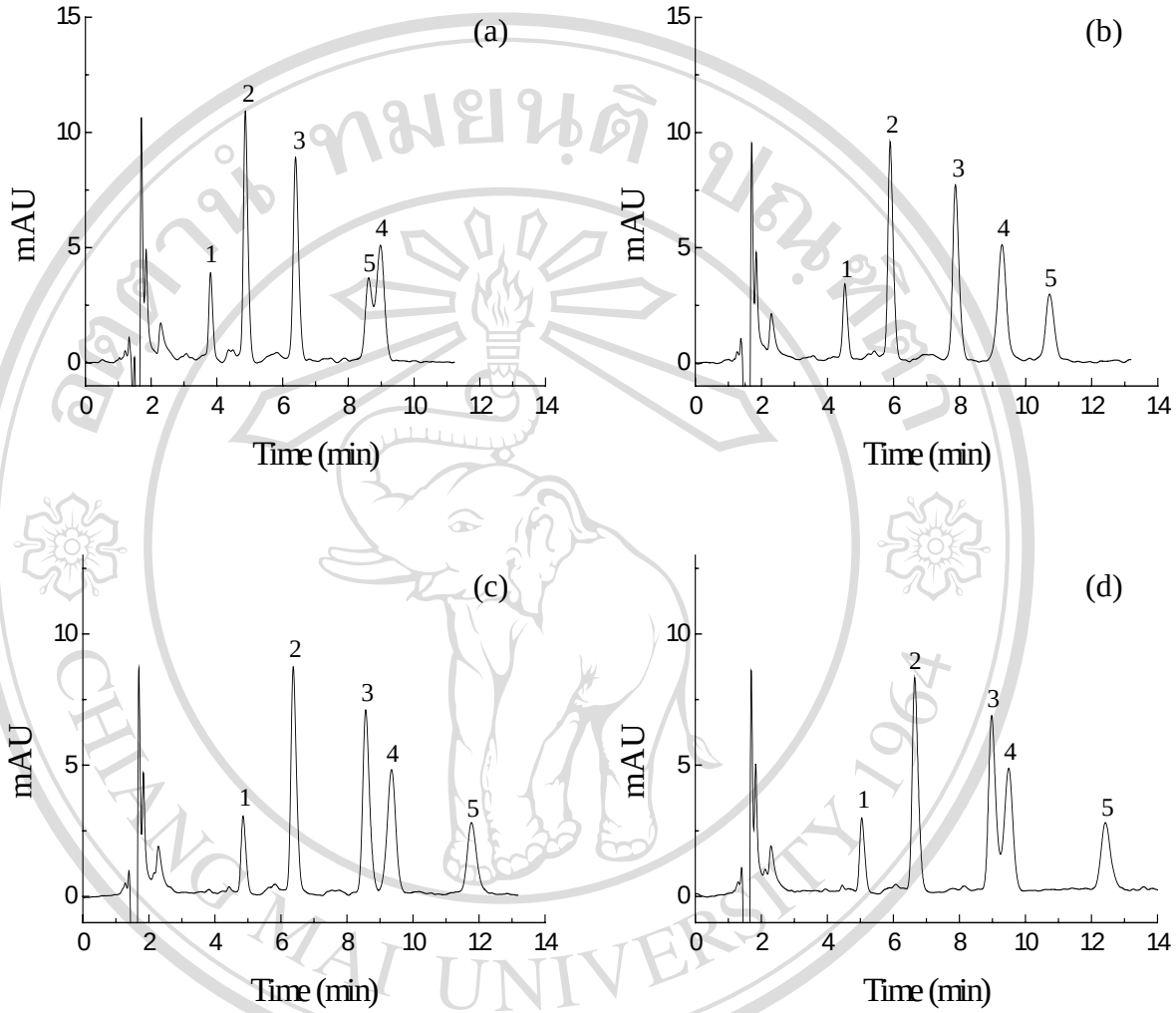

Fig. 3.11 Chromatograms of mixture of LAS compounds and Triton X-100 obtained using 75% (v/v) methanol in water containing various concentrations of ammonium acetate: (a) 1 mM; (b) 2 mM; (c) 3 mM; (d) 4 mM.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) Triton X-100; (5) C_{13} LAS.

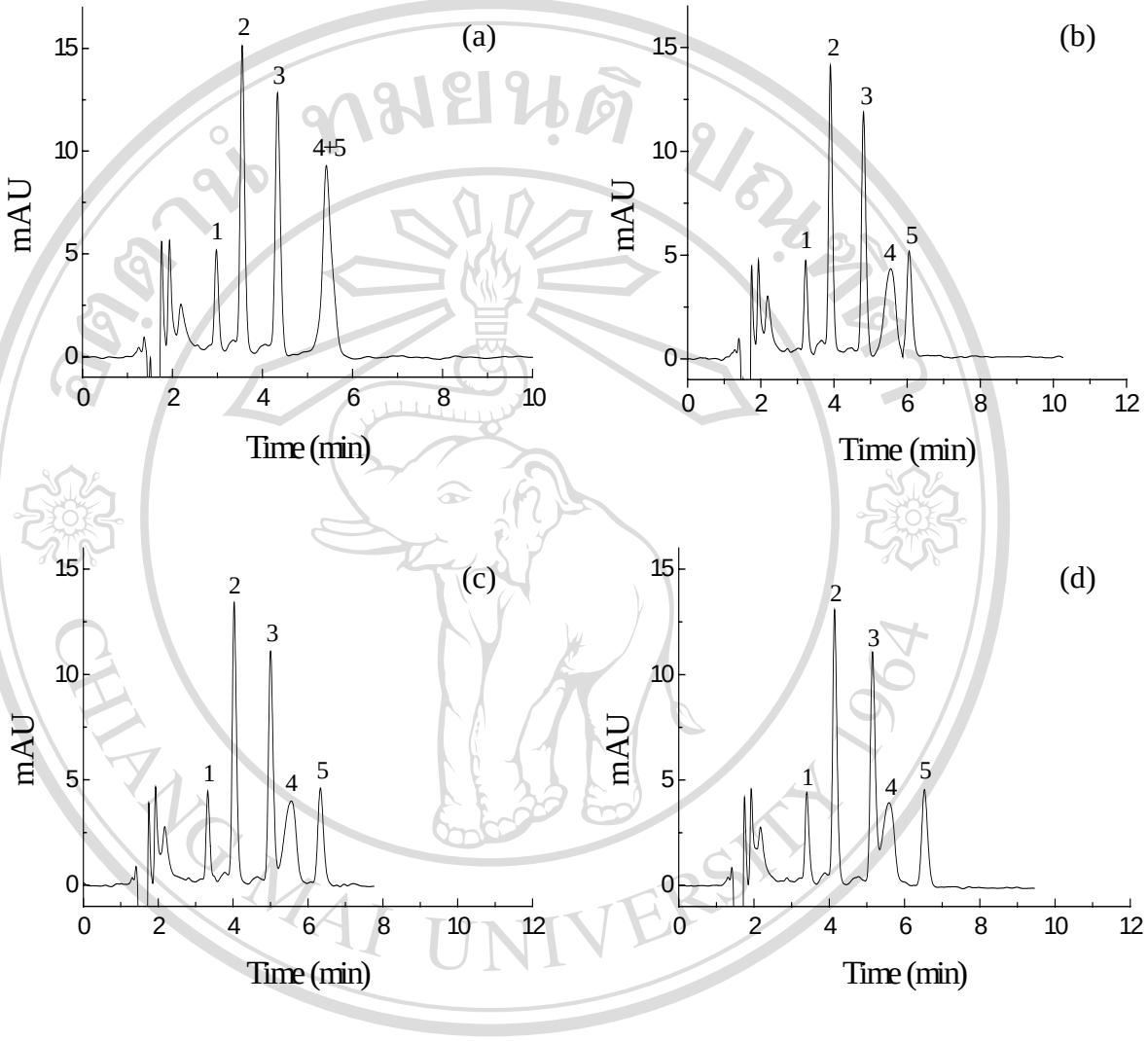

Fig. 3.12 Chromatograms of mixture of LAS compounds and Triton X-100 obtained using 80% (v/v) methanol in water containing various concentrations of ammonium acetate: (a) 2 mM; (b) 3 mM; (c) 4 mM; (d) 5 mM.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) Triton X-100; (5) C_{13} LAS.

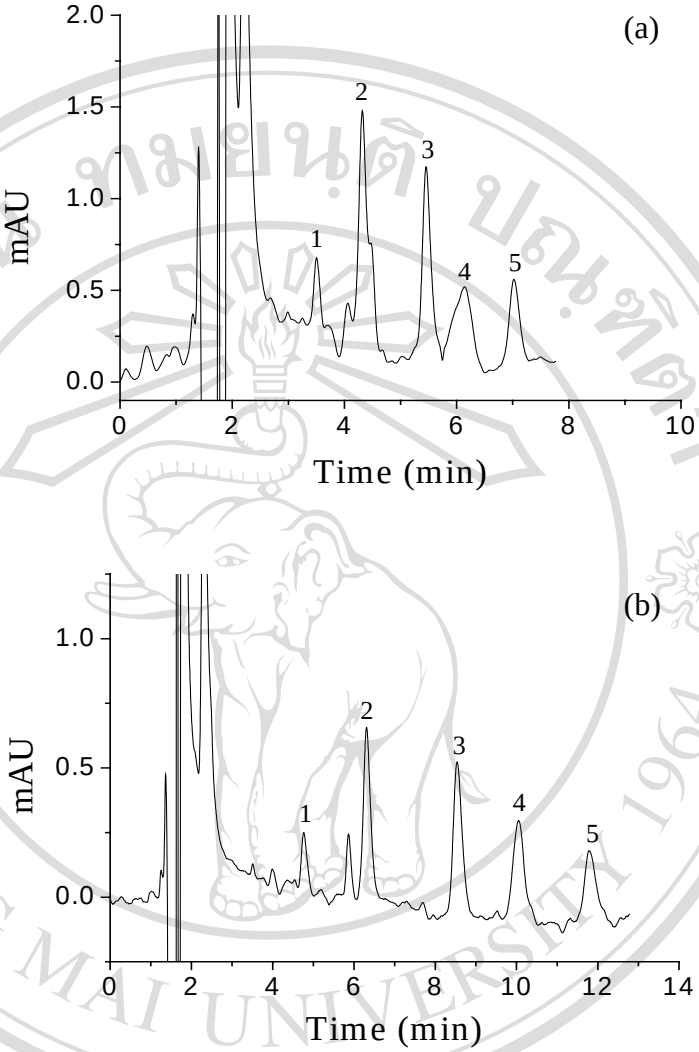

Fig. 3.13 Chromatograms of mixture of LAS compounds (0.2 ppm) and Triton X-100 (0.5 ppm) obtained using

(a) 80% (v/v) methanol in water containing 3 mM ammonium acetate and

(b) 75% (v/v) methanol in water containing 2 mM ammonium acetate.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) Triton X-100; (5) C_{13} LAS.

3.2.1.3 Effect of mobile phase flow rate

The optimum mobile phase composition obtained in Section 3.2.1.2 was used. The optimization of mobile phase flow rate was carried between 0.8 and 1.2 ml min^{-1}. The results were shown in **Table 3.2** and **Fig. 3.14**. Increasing flow rate affected to decrease resolution, analysis time and sensitivity. Although a decrease in resolution of each pair of neighboring surfactant compounds was observed, all conditions provided resolution > 1.5 for all neighboring surfactant peaks. Therefore, the analysis time and sensitivity would be the key factors for selecting an appropriate flow rate. The flow rate of 1.0 ml min^{-1}, instead of 0.8 ml min^{-1}, was chosen for the separation of LAS homologues and Triton X-100.

Table 3.2 Retention times and peak areas of each compound at various flow rates

Compound	Flow rate (ml min^{-1})					
	Retention time (min)			Peak area (mAU*s)		
	0.8	1.0	1.2	0.8	1.0	1.2
C_{10} LAS	5.666	4.532	3.766	39.03	34.50	26.21
C_{11} LAS	7.416	5.900	4.899	124.00	109.15	82.19
C_{12} LAS	9.957	7.866	6.524	126.87	114.30	84.27
C_{13} LAS	13.614	10.703	8.885	67.43	66.53	41.99
Triton X-100	11.749	9.277	7.703	107.20	104.41	73.02

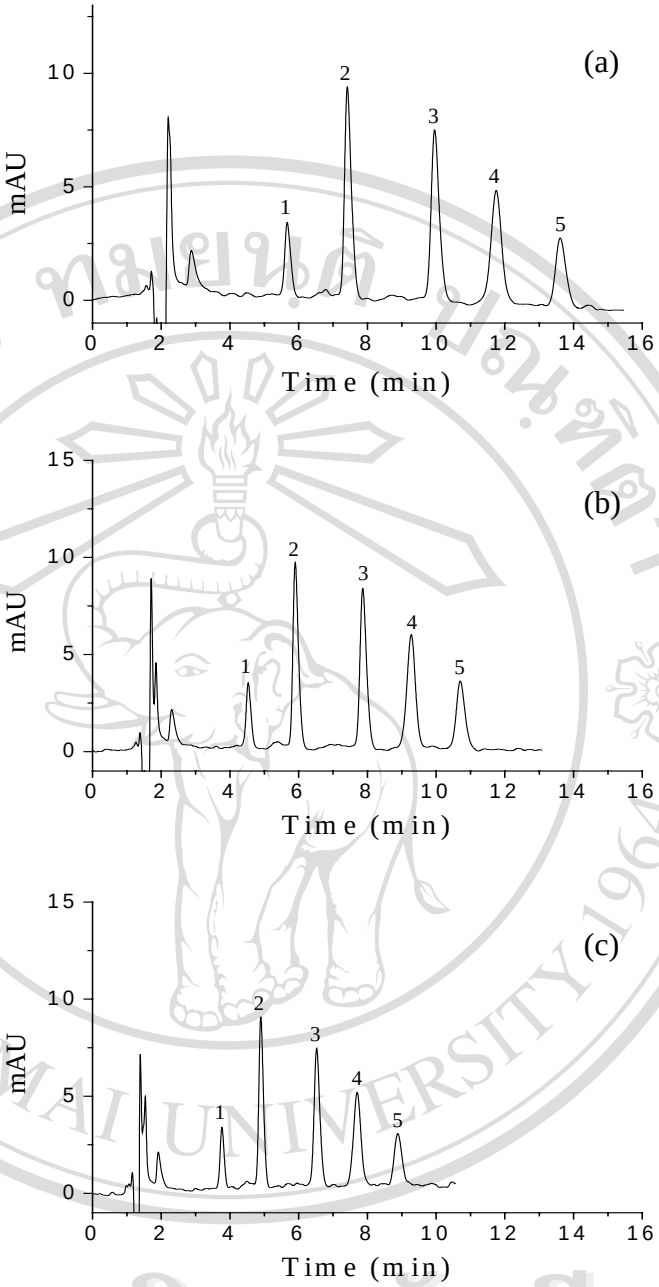

Fig. 3.14 Chromatograms of mixture of LAS compounds and Triton X-100 obtained using 75% (v/v) methanol in water containing 2 mM ammonium acetate at various flow rates: (a) 0.8 ml min^{-1}; (b) 1.0 ml min^{-1}; (c) 1.2 ml min^{-1}.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) Triton X-100; (5) C_{13} LAS.

3.2.2 Precision

The precision, repeatability and reproducibility give a measure of error in the development methodology and usually reported as a percentage of relative standard deviation (% R.S.D.). In this study, the repeatability was determined by eleven injections of a standard mixture onto C_8 column under the optimum conditions in same day. The reproducibility was determined by seven injections in different day. Results are shown in **Tables 3.3-3.6**. The repeatability of the retention time and peak area of each compound expressed as R.S.D. were found to be 0.9-1.3% and 0.6-1.7%, respectively. The reproducibility of the retention time and peak area obtained were 1.4-2.4% and 2.4-3.4%, respectively. These values are considered acceptable for analytical purposes as it indicates < 5% (high precision).

Table 3.3 Repeatability of the retention time of each compound

No. of injection	Retention time (min)				
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS	Triton X-100
1	4.659	6.121	8.222	11.276	9.444
2	4.562	5.977	8.046	11.126	9.352
3	4.693	6.177	8.325	11.426	9.576
4	4.591	6.025	8.084	11.049	9.384
5	4.645	6.126	8.266	11.370	9.549
6	4.611	6.047	8.116	11.100	9.415
7	4.557	5.989	8.092	11.188	9.455
8	4.654	6.124	8.251	11.302	9.555
9	4.577	6.005	8.092	11.201	9.474
10	4.679	6.163	8.302	11.371	9.620
11	4.549	5.969	8.046	11.129	9.442
average	4.616	6.066	8.167	11.231	9.479
S.D.	0.05	0.08	0.11	0.13	0.08
% R.S.D.	1.1	1.3	1.3	1.1	0.9

Table 3.4 Repeatability of the peak area of each compound

No. of injection	Peak area (mAU*s)				
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS	Triton X-100
1	30.69	96.63	97.53	50.33	84.48
2	31.30	96.55	96.60	50.17	84.17
3	31.44	96.80	97.86	49.67	86.19
4	31.43	96.73	98.27	51.03	83.20
5	31.15	95.94	97.23	49.23	84.77
6	31.63	96.47	96.66	49.18	83.42
7	31.50	96.27	98.64	51.01	86.46
8	31.56	95.45	96.27	49.10	83.34
9	31.13	96.74	96.38	50.43	85.35
10	30.57	96.31	98.78	51.82	84.10
11	30.91	94.94	95.73	50.38	85.32
average	31.21	96.26	97.27	50.21	84.62
S.D.	0.36	0.59	1.03	0.87	1.12
% R.S.D.	1.1	0.6	1.1	1.7	1.3

Table 3.5 Reproducibility of the retention time of each compound

No. of injection	Retention time (min)				
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS	Triton X-100
1	4.616	6.066	8.167	11.231	9.479
2	4.612	6.061	8.171	11.290	9.781
3	4.527	5.897	7.873	10.722	9.284
4	4.532	5.900	7.866	10.703	9.277
5	4.484	5.871	7.877	10.781	9.679
6	4.463	5.834	7.818	10.687	9.637
7	4.479	5.857	7.857	10.752	9.676
average	4.530	5.927	7.947	10.881	9.545
S.D.	0.06	0.10	0.15	0.26	0.20
% R.S.D.	1.4	1.6	1.9	2.4	2.1

Table 3.6 Reproducibility of the peak area of each compound

No. of injection	Peak area (mAU*s)				
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS	Triton X-100
1	31.21	96.26	97.27	50.21	84.62
2	29.89	98.49	98.01	51.16	90.67
3	31.14	99.22	99.86	51.17	89.51
4	32.42	103.27	104.22	54.11	94.47
5	31.06	96.94	97.88	50.19	89.95
6	31.10	97.40	97.46	51.79	88.08
7	31.13	97.42	97.52	52.08	87.83
average	31.14	98.43	98.89	51.53	89.30
S.D.	0.73	2.35	2.51	1.34	3.02
% R.S.D.	2.4	2.4	2.5	2.6	3.4

3.2.3 Detection limit

The detection limit values were calculated from the linear regression line of the calibration curves as shown in **Figs. 3.15-3.19** based on a method of Miller and Miller (For a detailed calculation, see **Appendix**). The detection limits of four LAS compounds and Triton X-100 are summarized in **Table 3.7**.

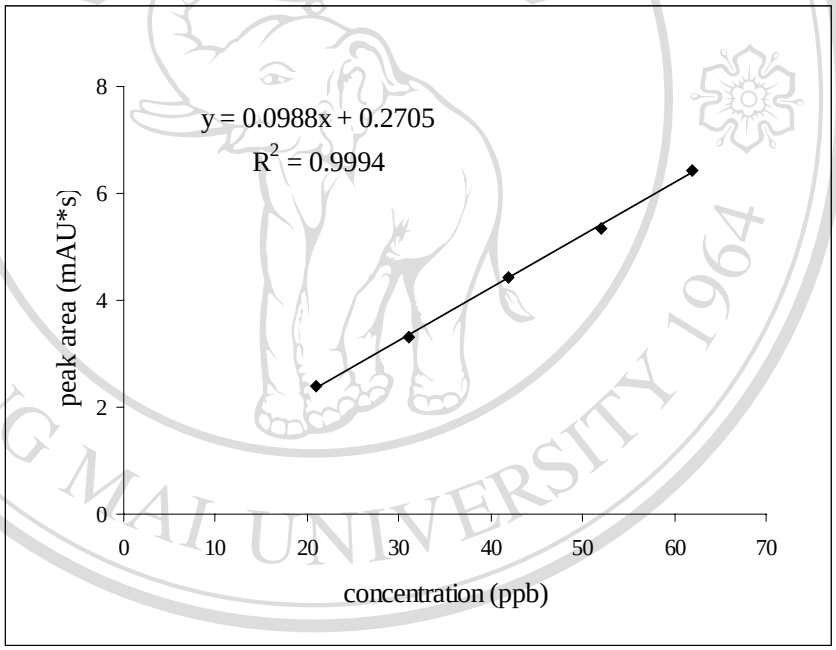

Fig. 3.15 Calibration curve of C_{10} LAS used for calculating the detection limit.

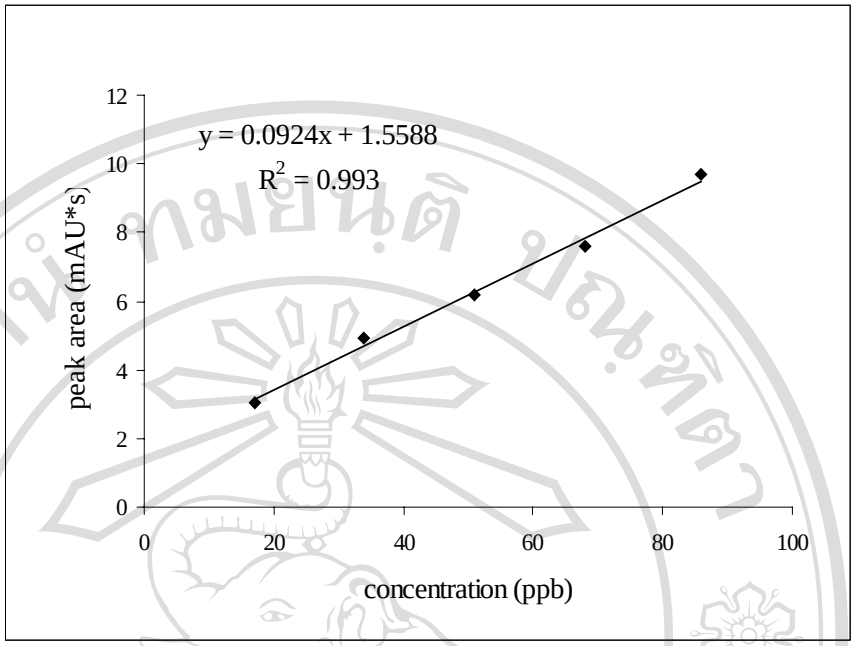

Fig. 3.16 Calibration curve of C_{11} LAS used for calculating the detection limit.

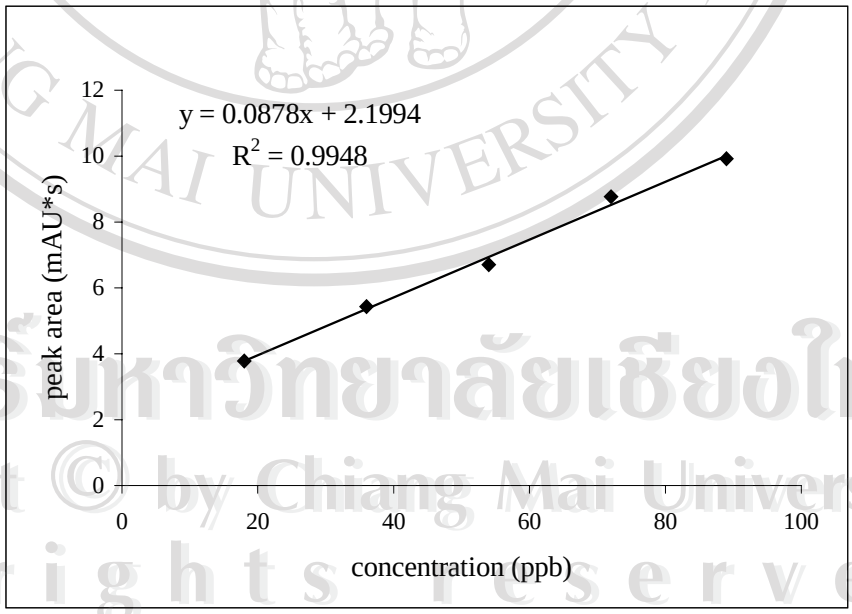

Fig. 3.17 Calibration curve of C_{12} LAS used for calculating the detection limit.

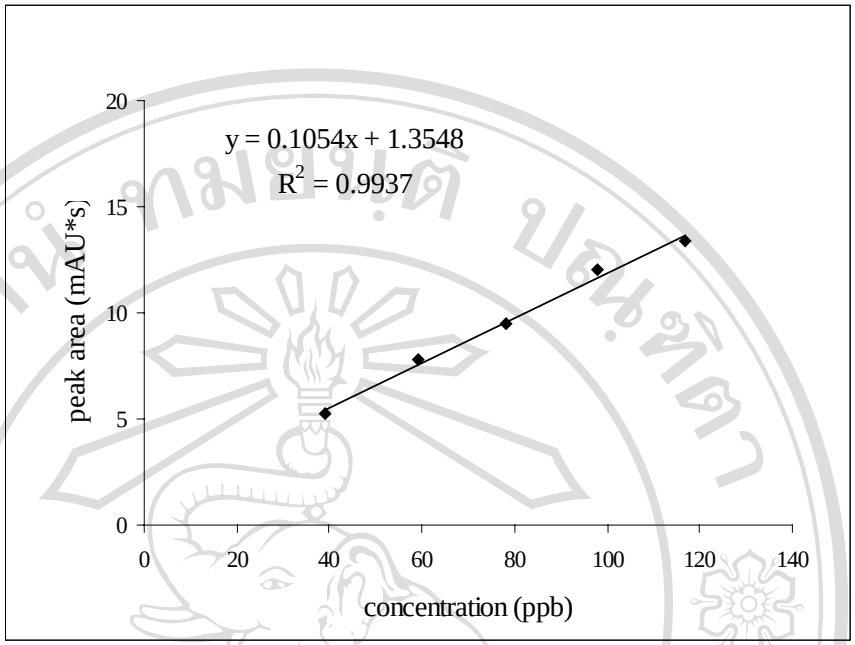

Fig. 3.18 Calibration curve of C_{13} LAS used for calculating the detection limit.

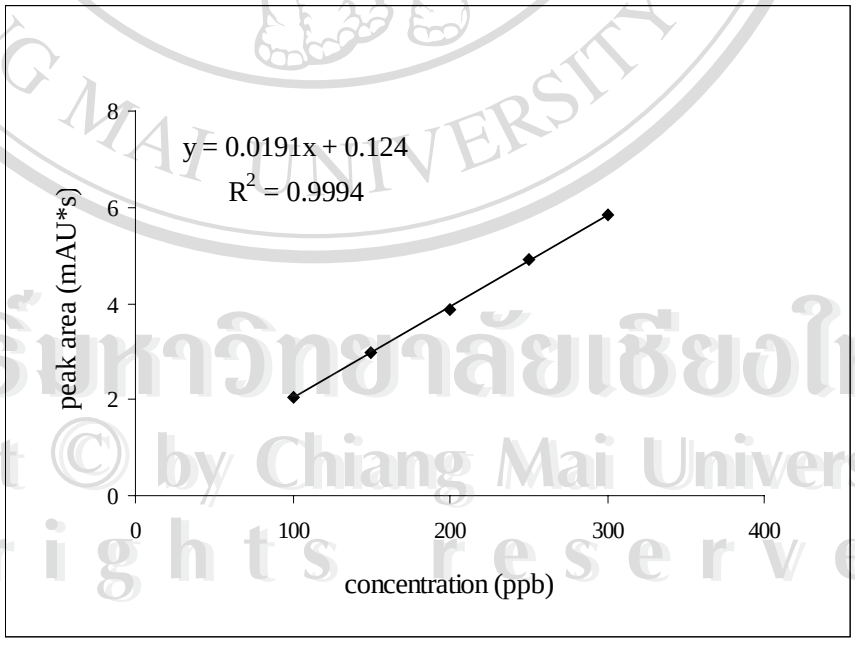

Fig. 3.19 Calibration curve of Triton X-100 used for calculating the detection limit.

Table 3.7 Summary of detection limit of each surfactant

Compound	Concentration range (ppb)	Regression line equation	Correlation coefficient (R^2)	Detection limit (ppb)
C_{10} LAS	21-62	y = 0.0988x + 0.2705	0.9994	1.4
C_{11} LAS	17-86	y = 0.0924x + 1.5588	0.9930	7.9
C_{12} LAS	18-89	y = 0.0878x + 2.1994	0.9948	7.1
C_{13} LAS	39-117	y = 0.1054x + 1.3548	0.9937	8.4
Triton X-100	100-300	y = 0.0191x + 0.1240	0.9994	28.5

3.2.4 SPE for LAS and Triton X-100 analysis

For environmental samples, the low concentrations in which anionic and nonionic surfactants are generally found. It is necessary to perform an initial stage of concentration and purification of the analytes prior to analysis. Solid-phase extraction was used for purification and preconcentration the surfactants.

The optimization of eluent volume was done by the method in Section 2.3.2.4. Peak areas of each compound obtained from different fractions of eluate are shown in **Table 3.8** and the elution profiles are shown in **Fig. 3.20**. It was found that the highest amounts of all LAS compounds and Triton X-100 were eluted in the third and the fourth fraction, respectively (**Fig. 3.20**). LAS compounds and Triton X-100 were completely eluted with 3 ml methanol.

Table 3.8 Effect of eluent volume on desorption of LAS compounds and Triton X-100

No. of fraction	Eluent volume (ml)	Peak area (mAU*s)				
		C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{12} LAS	Triton X-100
1	0.5	n.d.	2.54	5.33	n.d.	n.d.
2	1.0	2.22	6.69	9.86	3.05	n.d.
3	1.5	12.67	40.93	52.13	33.99	19.91
4	2.0	2.97	10.13	12.69	9.15	21.67
5	2.5	1.85	8.40	11.63	8.36	n.d.
6	3.0	n.d.	1.35	3.51	n.d.	n.d.
7	3.5	n.d.	n.d.	n.d.	n.d.	n.d.
8	4.0	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected.

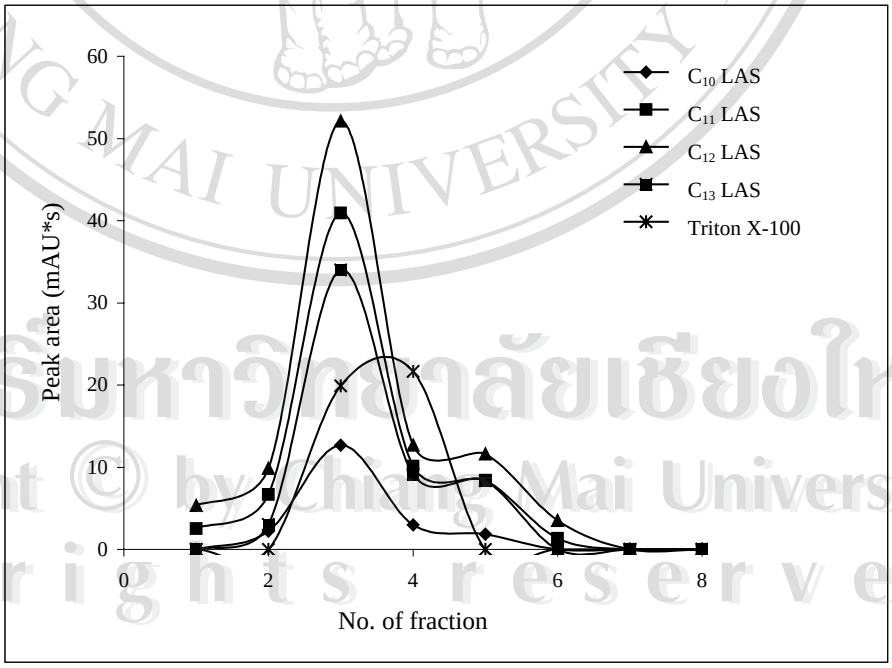

Fig. 3.20 Elution profiles of LAS compounds and Triton X-100 obtained using C_{18} cartridge.

3.2.5 Recovery test

Triplicate extraction of water samples was carried out by spiking each sample with mixed LAS compounds (2 ppm) and Triton X-100 (4 ppm). The results are shown in **Table 3.9**.

Table 3.9 The percentage recovery of extraction for water samples (n=3)

Type of water	% Recovery				
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS	Triton X-100
W1	99 (3.7)	88 (7.6)	90 (3.0)	97 (4.3)	96 (1.2)
W2	97 (1.3)	98 (2.6)	96 (1.5)	94 (1.9)	99 (1.3)
W3	96 (1.9)	90 (2.4)	82 (7.6)	95 (5.1)	91 (3.4)
W4	87 (1.6)	101 (3.5)	98 (3.8)	93 (4.0)	99 (0.5)
W5	86(4.3)	96(3.9)	93(5.2)	88(7.6)	92(2.8)
W6	86 (9.2)	84 (3.2)	87 (3.6)	100 (7.9)	97 (2.2)
W7	96 (1.4)	97 (2.4)	89 (2.2)	94 (5.8)	98 (2.6)
W8	97 (0.8)	106 (3.6)	112 (2.3)	123 (2.4)	96 (4.5)
W9	98 (1.6)	96 (3.0)	98 (2.8)	103 (2.4)	101 (2.4)

Number in parentheses corresponds to % relative standard deviation.

W1, drainage water from student dormitory, Chiang Mai University; W2, wastewater from Center of Medical Sciences, Ministry of Public Health, Chiang Mai; W3, wastewater from Khuy hospital, Utraradit; W4, wastewater in Mae-Kha canal, Chiang Mai; W5, domestic wastewater released into Thorn canal, Utraradit; W6, natural water in Mae-Ping river, Chiang Mai, W7, natural water from irrigation canal, Chiang Mai; W8, natural water in Ang-Kaew reservoir, Chiang Mai University; W9, water in Chiang Mai moat, Chiang Mai.

The accuracy expressed in terms of percentage recovery was done by spiking the mixture of LAS compounds and Triton X-100 into various water samples. The percentage recoveries of this proposed method for C_{10} LAS, C_{11} LAS, C_{12} LAS, C_{13} LAS and Triton X-100 were 86-99, 84-106, 82-112, 88-123 and 91-101, respectively. Satisfactory recovery was obtained, particularly C_{13} LAS comparing to those reported elsewhere [42, 43].

3.2.6 Analysis of LAS and Triton X-100 in water samples

3.2.6.1 Determinations of LAS and Triton X-100 in real water samples using HPLC-UV

In order to demonstrate the suitability of the proposed method in this study for quantification of LAS and Triton X-100 in real samples, several natural water and wastewater extracts were analysed. Calibration curves based on peak area were linear (R^2 = 0.9979-0.9997) for each surfactant compound in the range test 0.01-0.31 ppm for C_{10} LAS, 0.02-1.03 ppm for C_{11} LAS, 0.02-1.07 ppm for C_{12} LAS, 0.01-0.59 ppm for C_{13} LAS and 0.5-4 ppm for Triton X-100 as illustrated in **Figs. 3.21-3.25**. C_{11} LAS and C_{12} LAS were found to be the major compounds present in all the wastewater samples examined (**Fig. 3.26**). In natural water samples, no LAS homologues (except sample W7) and Triton X-100 were detected. Under the proposed condition, the LAS compounds 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 3.10**.

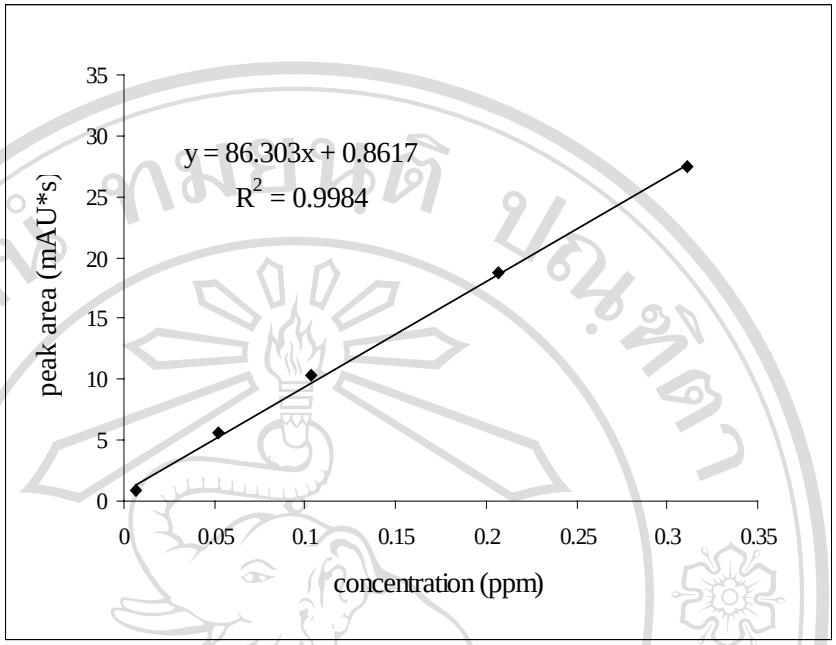

Fig. 3.21 Calibration curve of C_{10} LAS used for calculating concentration in water samples.

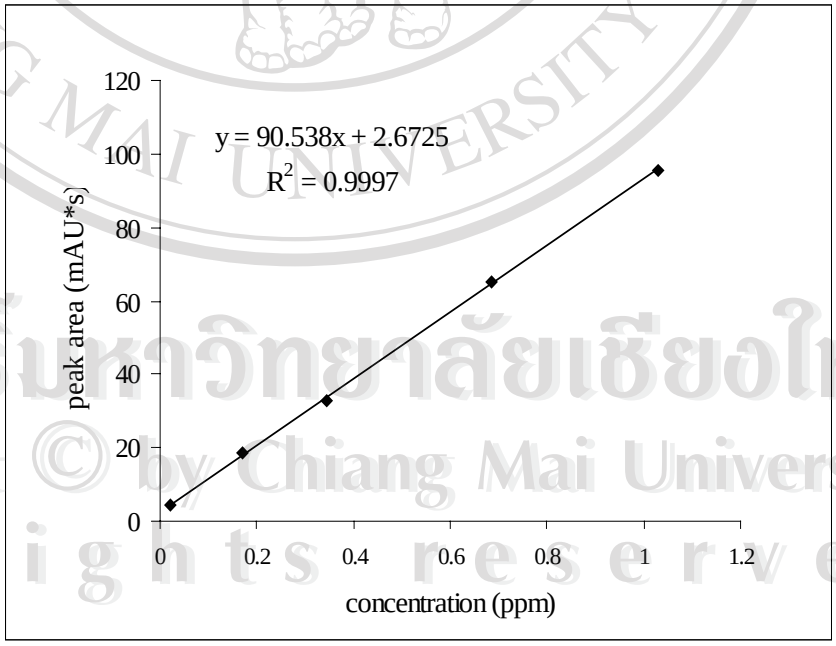

Fig. 3.22 Calibration curve of C_{11} LAS used for calculating concentration in water samples.

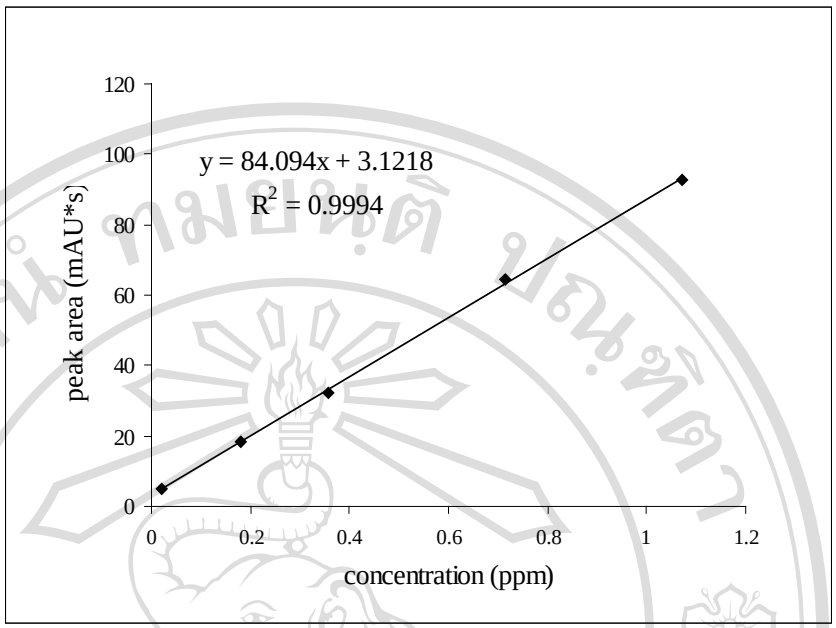

Fig. 3.23 Calibration curve of C_{12} LAS used for calculating concentration in water samples.

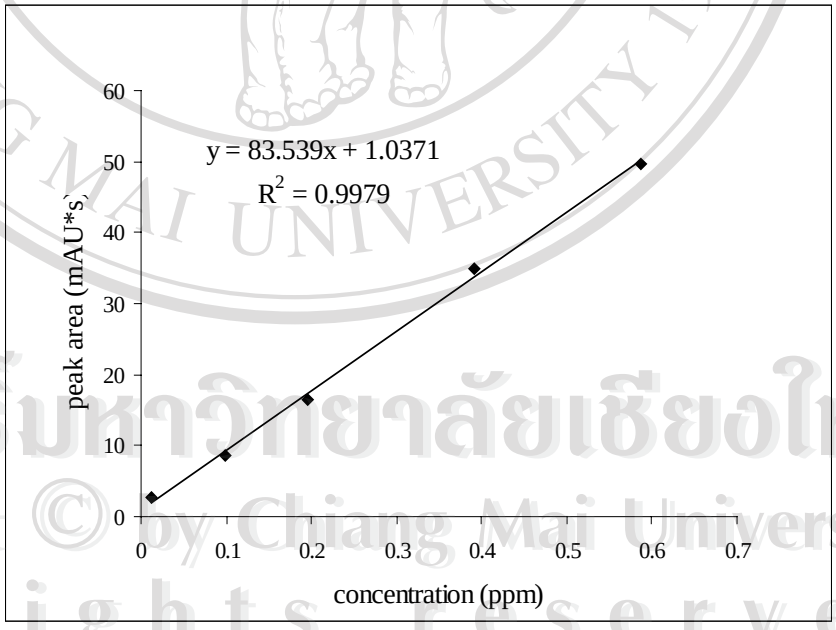

Fig. 3.24 Calibration curve of C_{13} LAS used for calculating concentration in water samples.

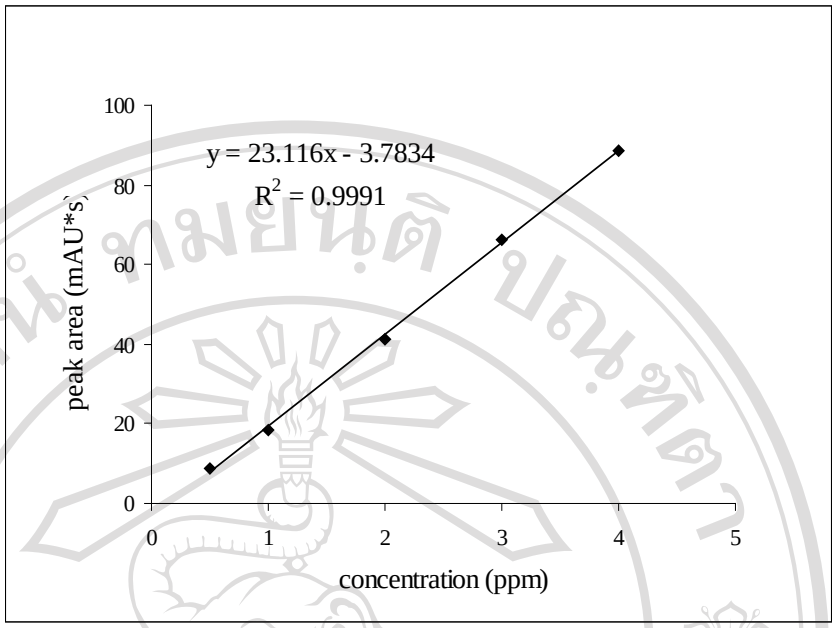

Fig. 3.25 Calibration curve of Triton X-100.

Table 3.10 Amounts of LAS compounds and Triton X-100 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	Triton X-100
W1	115.3 ± 2.7	303.2 ± 7.9	184.4 ± 8.0	81.9 ± 2.2	n.d.
W2	310.1 ± 0.3	1173.4 ± 4.6	1145.0 ± 12.9	423.4 ± 5.0	n.d.
W3	5.0 ± 0.1[a]	23.4 ± 0.5	26.5 ± 1.1	21.1 ± 1.5	n.d.
W4	54.1 ± 1.9	120.1 ± 5.5	69.1 ± 4.8	36.6 ± 5.2	n.d.
W5	n.d.	n.d.	13.0 ± 0.9	14.8 ± 0.5	n.d.
W6	n.d.	n.d.	n.d.	n.d.	n.d.
W7	3.4 ± 0.1[a]	12.8 ± 0.9	15.1 ± 1.3	16.7 ± 1.6	n.d.
W8	n.d.	n.d.	n.d.	n.d.	n.d.
W9	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected (less than the detection limit value).

[a] Preconcentration as described in Section 2.3.2.5

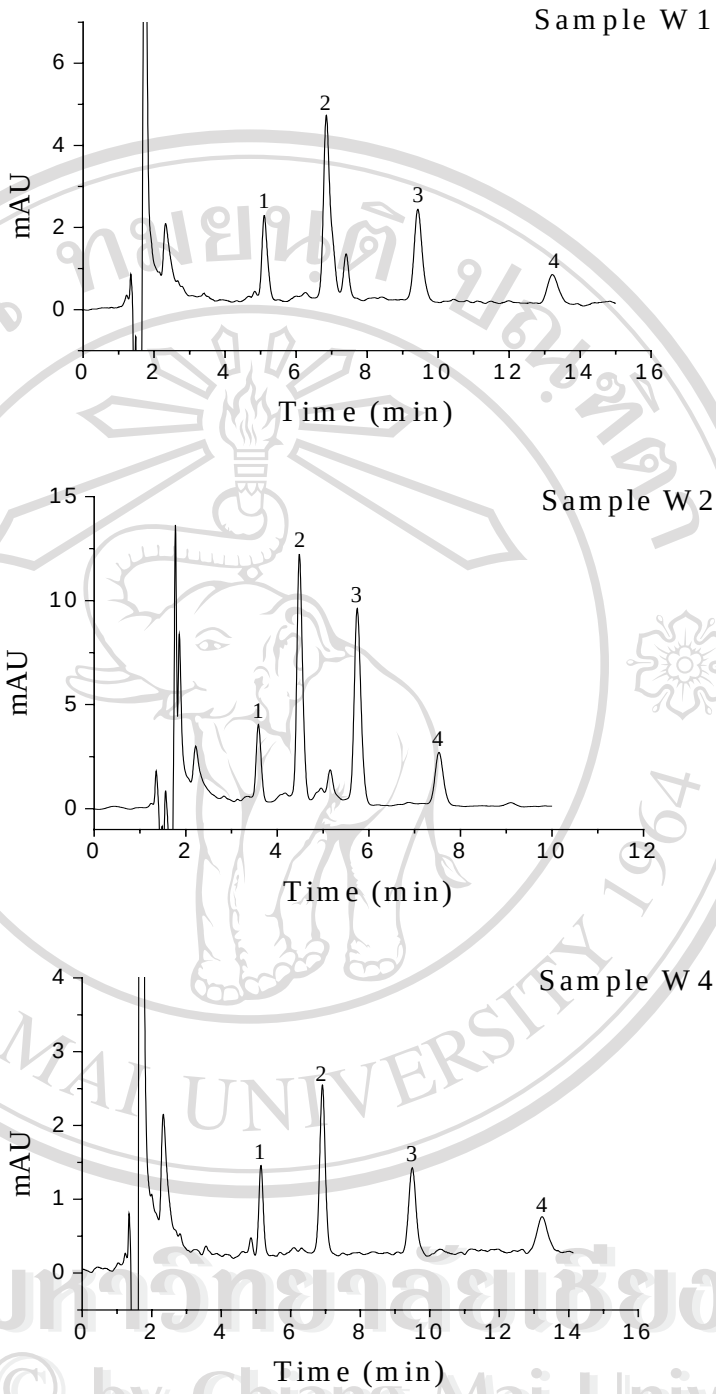

Fig. 3.26 Chromatograms of mixture of four LAS compounds in water extracts obtained using 74% (v/v) methanol in water (for sample W1 and W4) and 77% (v/v) methanol in water (for sample W2) containing 2 mM ammonium acetate. Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

3.2.6.2 Identification of LAS compounds in water extracts 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 [37, 54].

To overcome such problems, the negative-ion electrospray (ES)-mass spectrometry (MS) was used for confirmation of LAS compounds in water samples. The total ion chromatograms (TIC) and mass spectra of some water extracts were showed in **Figs 3.27** and **3.28-3.30**, respectively. The mass spectra of water extracts showed the *m/z* 183 ion common to LAS compounds. In addition, the high intensity of the pseudomolecular ions, corresponding to $[M\text{-}Na]^-$ observed at *m/z* 297, 311, 325 and 339 originating from the water extracts are similar to those originating from the LAS standards (**Fig. 3.8**). These ions corresponding to C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS, respectively.

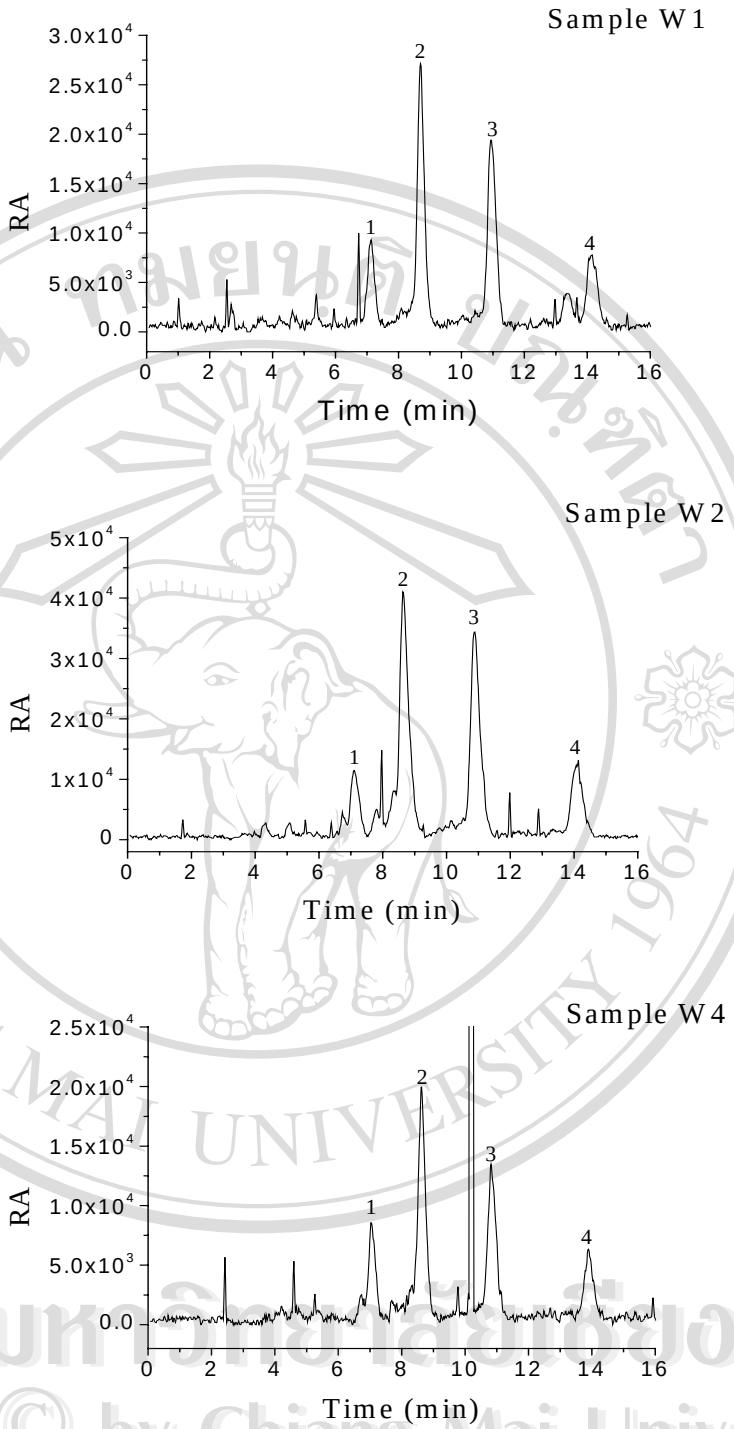

Fig. 3.27 Total ion chromatograms (TIC) of water extracts W1, W2 and W4 obtained using 80% (v/v) methanol in water containing 1.5 mM ammonium acetate, showed the presence of LAS compounds.

Peak identification: (1) C_{10} LAS; (2) C_{11} LAS; (3) C_{12} LAS; (4) C_{13} LAS.

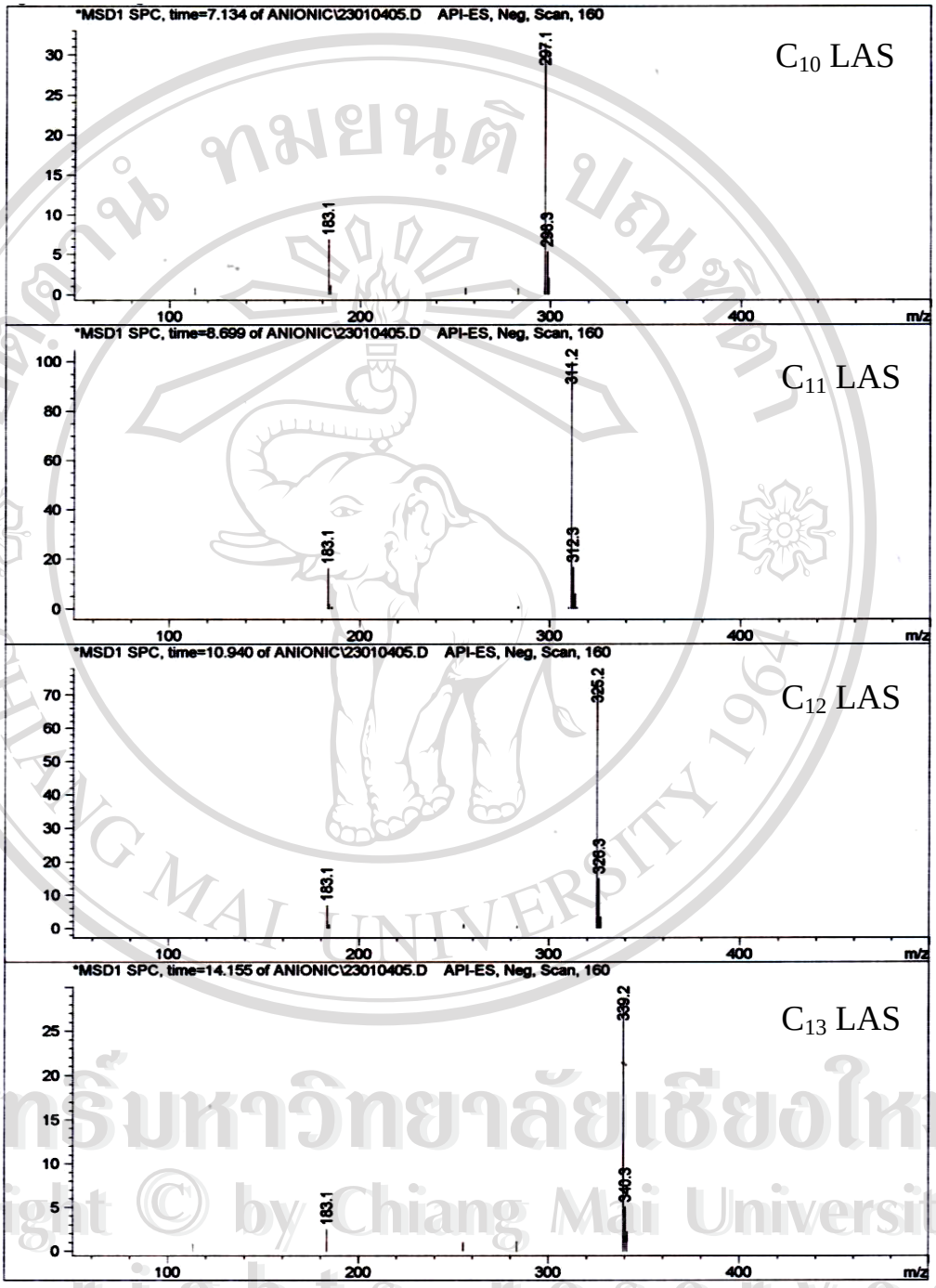

Fig. 3.28 Negative-ion ES mass spectra of LAS compounds originating from water extract W1.

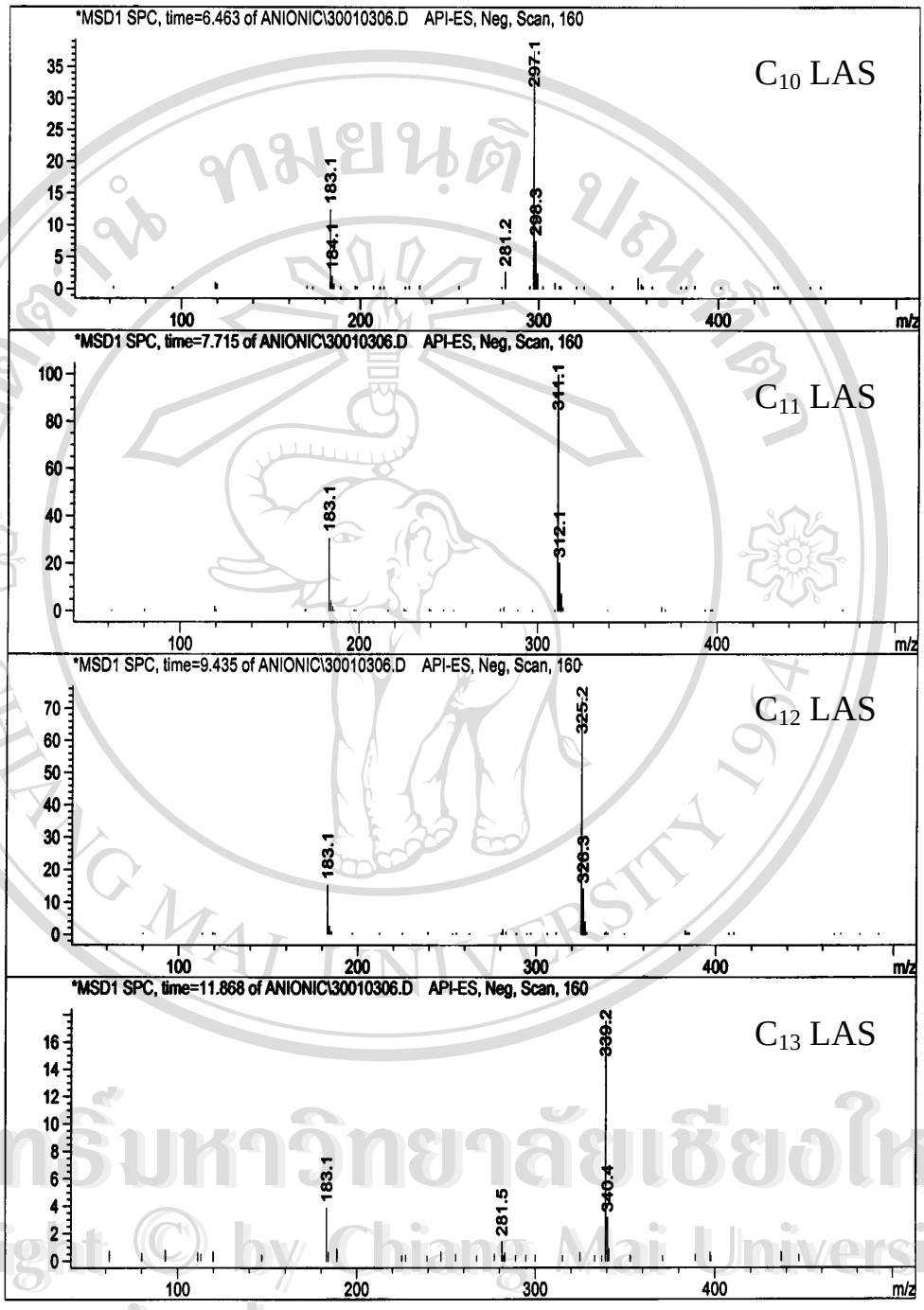

Fig. 3.29 Negative-ion ES mass spectra of LAS compounds originating from water extract W2.

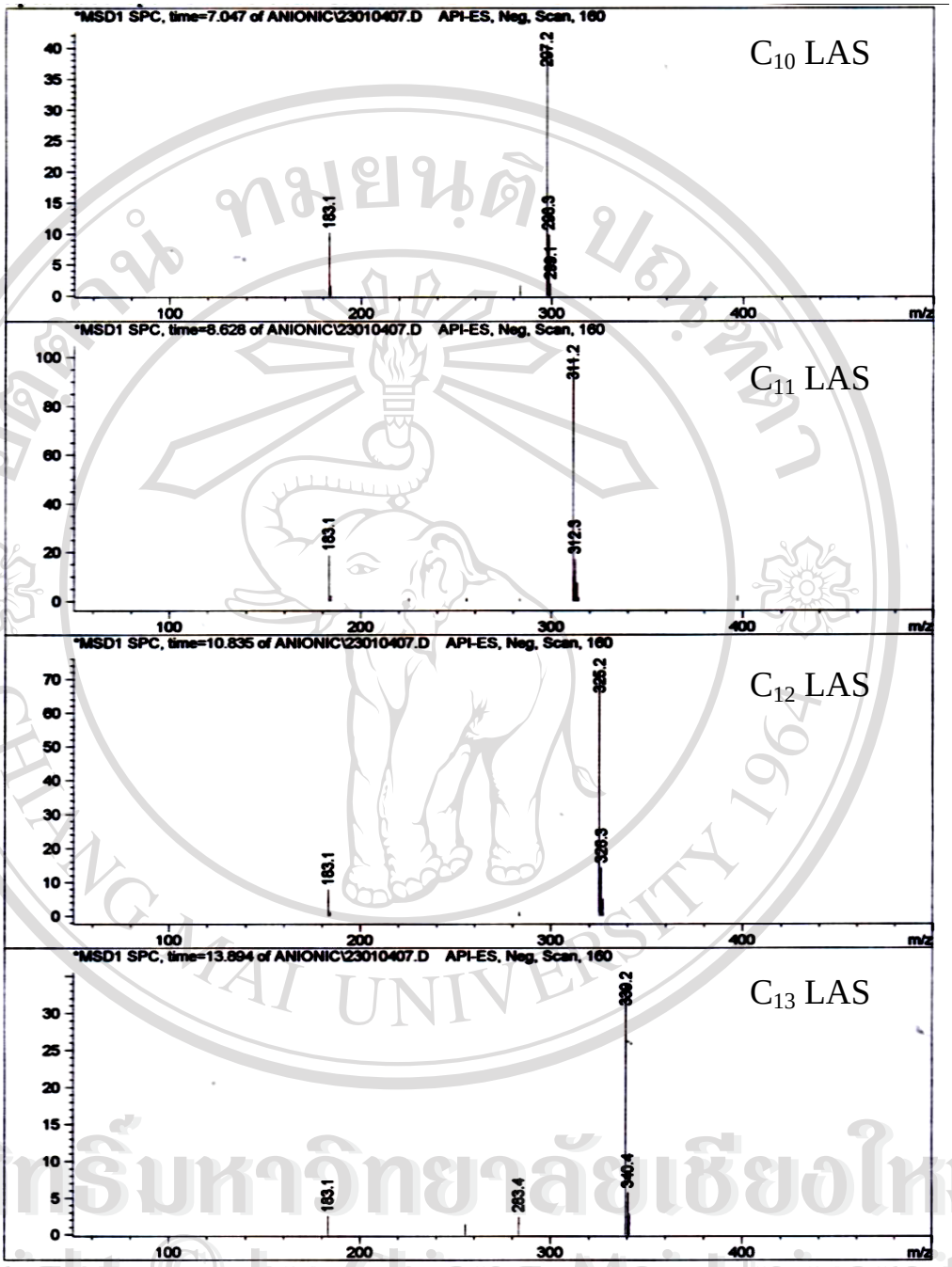

Fig. 3.30 Negative-ion ES mass spectra of LAS compounds originating from water extract W4.

3.3 Analysis of SDS

3.3.1 Optimization of HPLC conditions

SDS is a compound which can not absorb UV light. It is therefore not detected using UV detection. In this work, SDS was analysed using HPLC-UV by indirect detection, adding chromophore, i.e. lidocaine to mobile phase.

3.3.1.1 Effect of detection wavelength

The detection wavelength was determined in the range of 230-260 nm by injecting 20 µl of 10 ppm of SDS standard. The mobile phase used was 40% methanol in water containing 1 ppm lidocaine at pH 3.0. Results were shown in **Table 3.11**. The maximum detector response expressed as peak area was obtained at 230 nm. Therefore, this wavelength was employed for the further method development.

Table 3.11 Peak areas of SDS at various wavelengths

Wavelength (nm)	Peak area of SDS (mAU*s)
230	39.98
240	14.35
250	4.76
260	5.56

3.3.1.2 Effect of mobile phase composition

The mixture of methanol and water containing 1 ppm lidocaine at pH 3.0 was employed in this study. Mobile phase compositions in the range of 30-50% methanol in water were investigated. A 20 µl of 10 ppm of SDS standard was injected. Results were shown in **Table 3.12** and **Fig. 3.31**. It was found that increasing the methanol concentrations resulted to reduce retention time of SDS peak. The repeatability of the retention time and peak area expressed as % R.S.D. were investigated by eleven injecting of SDS standard in all conditions for helping to select the suitable condition. The highest detector response was obtained when 30% methanol was used. However, % R.S.D. of the retention time and peak area was higher than 5%. This is considered unacceptable for analytical purposed, particularly retention time. Using 35% methanol, % R.S.D. of peak area was also higher than 5%. The retention time and peak area repeatability were found to be 2.4% and 3.8%, respectively when 40% methanol was used. Hence, 40% (v/v) methanol in water was selected for the further experiments.

Table 3.12 Retention times and peak areas of SDS standard as a function of mobile phase compositions (n = 11)

% MeOH	Retention time (min)	Peak area (mAU*s)
30	4.325 (5.3)	36.42 (6.2)
35	3.980 (2.6)	26.16 (5.9)
40	2.850 (2.4)	19.08 (3.8)
45	2.597 (2.0)	10.08 (2.5)
50	2.376 (1.6)	6.12 (7.3)

Number in parentheses corresponds to % relative standard deviation.

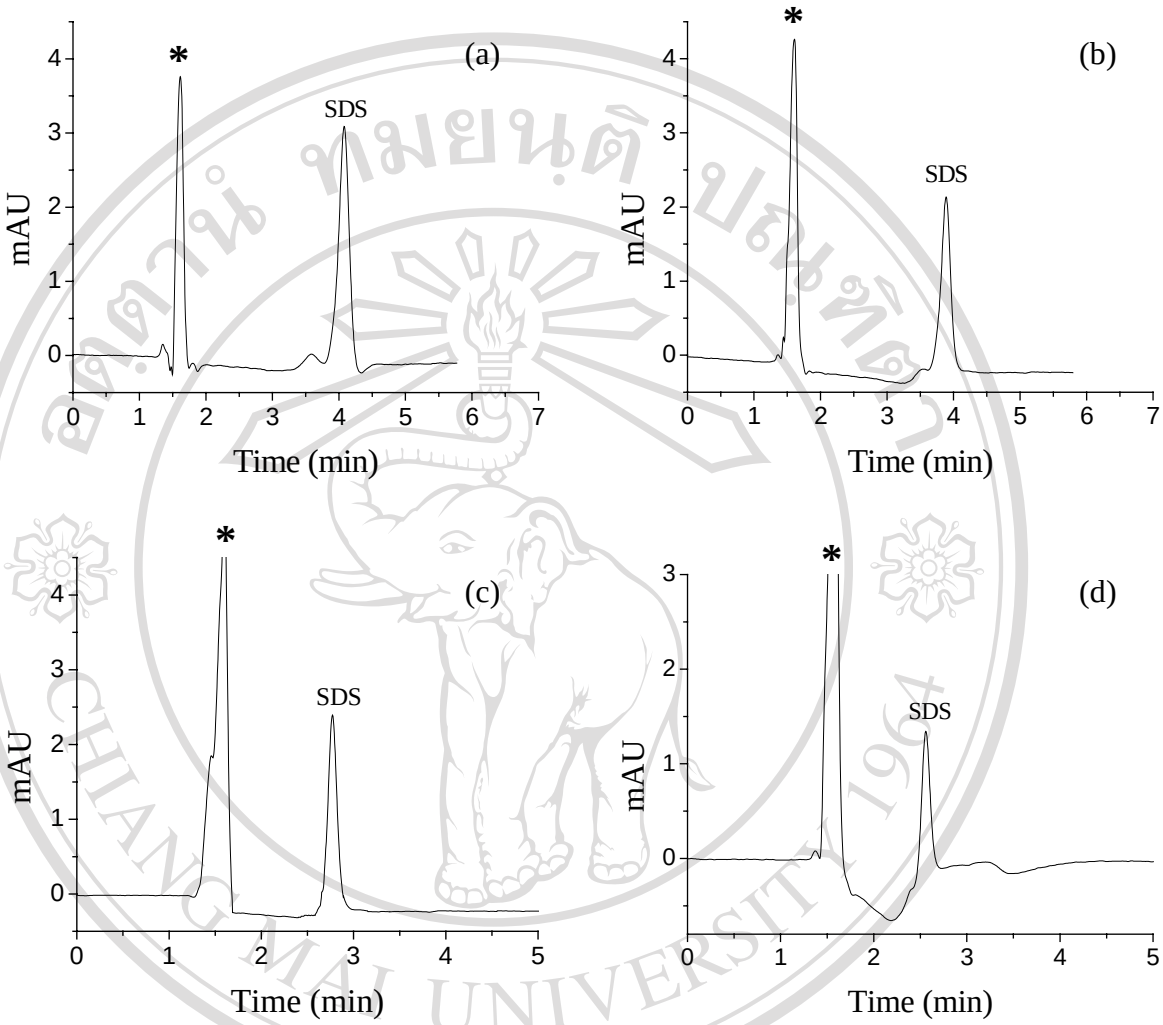

Fig. 3.31 Chromatograms of SDS standard obtained using various mobile phase compositions in the presence of 1 ppm lidocaine at pH 3.0 and MeOH-H_2O: (a) 30:70; (b) 35:65; (c) 40:60; (d) 45:55.

* Impurity due to the use of water in place of the mobile phase.

3.3.1.3 Effect of pH of mobile phase

Mobile phase consisting of 40% methanol and 1 ppm lidocaine in water was used for varying pH range from 3.0-4.0. From the results shown in **Table 3.13**, it was observed that the retention time of SDS did not depend on pH of mobile phase in the range 3.0-4.0. Lidocaine is protonated to positively charged at pH less than 7.9 because log K of lidocaine is 7.9 which K is protonation constant [55]. In addition, SDS is negatively charged specie at pH test [12]. It is evident from the results shown in **Table 3.13** that the pH of mobile phase affects peak area of SDS. R.S.D. values of retention time and peak area were higher than 5% when using pH of mobile phase exceeding 3.5. At pH 3.3 and 3.4, R.S.D. values of both of retention time and peak area were less than 5%. Difference in signal responses at both pH was not significant. So mobile phase at pH 3.4 was chosen in order to keep column's life.

Table 3.13 Retention times and peak areas of SDS standard as a function of mobile phase pH (n = 11)

pH	Retention time (min)	Peak area (mAU*s)
3.0	2.850 (2.4)	19.08 (3.8)
3.3	2.956 (4.2)	57.04 (4.9)
3.4	2.750 (4.8)	54.24 (3.5)
3.5	2.493 (5.2)	49.02 (5.6)
3.6	2.928 (6.5)	82.09 (6.8)
3.7	3.074 (7.9)	79.01 (9.6)
4.0	3.072 (6.3)	78.84 (20.2)

Number in parentheses corresponds to % relative standard deviation.

3.3.1.4 Effect of lidocaine concentration

The concentrations of lidocaine in 40% (v/v) methanol in water at pH 3.4 were examined in the range of 0.1-6.5 ppm. The results in **Table 3.14** showed that the concentration of lidocaine in mobile phase affected the peak area of SDS dramatically. As lidocaine is a chromophore, the selection of a suitable amount of lidocaine is a critical factor in obtaining a maximum response. Using lidocaine concentration exceeding 5.0 ppm, % R.S.D. of peak area was higher than 5%. Therefore, the concentration of lidocaine at 5 ppm was selected to add in mobile phase because it provided high sensitivity and also its R.S.D. value (< 5%) was acceptable for analytical method.

3.3.2 Summary of suitable HPLC conditions

The suitable mobile phase for the analysis of SDS was the mixture of 40% (v/v) methanol in water containing 5 ppm lidocaine at pH 3.4, the flow rate of 1.0 $ml.min^{-1}$ and the detection wavelength of 230 nm. The chromatogram obtained using 10 ppm of SDS standard in the mobile phase is shown in **Fig. 3.32**.

Under the suitable condition, the reproducibility of retention time and peak area for this compound determined seven injections was found to be 1.3 and 4.8, respectively (**Table 3.15**).

The detection limit value of SDS was calculated from the linear regression line of the calibration curve as shown in **Fig. 3.33** with the correlation coefficient (R^2) 0.9927. The detection limit calculated was 0.3 ppm which was much higher than that of four LAS compounds. This is the disadvantage of indirect UV detection [15].

Table 3.14 Retention times and peak areas of SDS standard as a function of lidocaine concentrations in mobile phase (n = 11)

Lidocaine concentration (ppm)	Retention time (min)	Peak area (mAU*s)
0.1	2.739 (3.9)	4.59 (3.8)
0.5	2.635 (2.2)	14.20 (4.4)
1.0	2.750 (2.7)	29.97 (4.6)
1.5	2.780 (3.1)	43.46 (4.5)
2.0	2.596 (2.7)	42.35 (4.0)
2.5	2.746 (3.4)	65.13 (3.0)
3.0	2.505 (2.4)	55.42 (4.2)
3.5	2.520 (2.8)	75.43 (4.5)
4.0	2.640 (2.5)	89.36 (4.2)
4.5	2.459 (2.2)	75.81 (4.5)
5.0	2.529 (2.7)	98.07 (4.7)
5.5	2.656 (2.8)	119.36 (6.0)
6.0	2.490 (2.2)	95.54 (7.2)
6.5	2.703 (2.6)	135.17 (7.2)

Number in parentheses corresponds to % relative standard deviation.

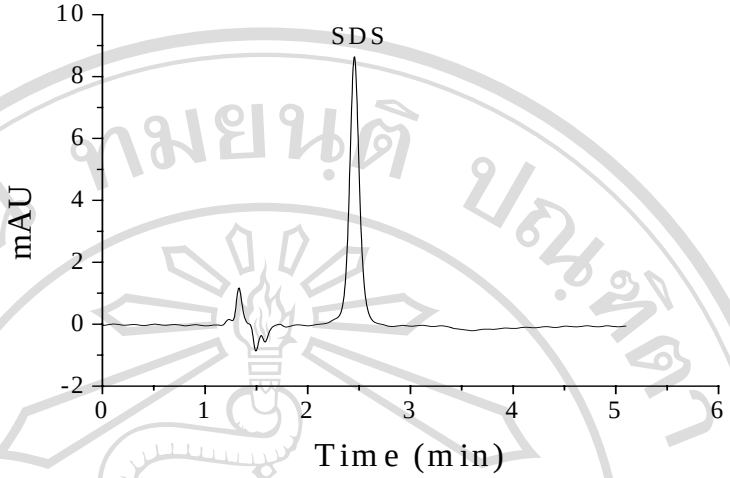

Fig. 3.32 Chromatogram of SDS standard under the suitable condition.

Table 3.15 Reproducibility of the retention time and peak area of SDS

No. of injection	Retention time (min)	Peak area (mAU*s)
1	2.498	89.73
2	2.527	93.49
3	2.504	91.54
4	2.537	83.87
5	2.554	84.20
6	2.598	92.63
7	2.548	84.57
average	2.538	88.58
S.D.	0.03	4.24
% R.S.D.	1.3	4.8

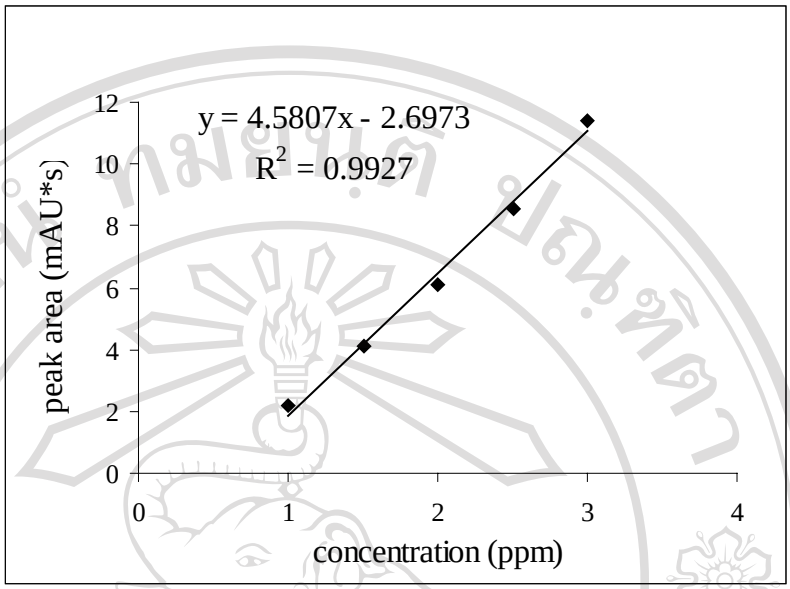

Fig 3.33 Calibration curve of SDS used for calculating the detection limit.

3.3.3 SPE for SDS analysis

Elution profile of SDS was investigated by using C_{18} cartridge. The optimization of eluent volume was done by the method in Section 2.3.3.4. Peak areas of SDS obtained from different fractions of eluate were shown in **Table 3.16** and the elution profile was shown in **Fig. 3.34**. It was found that the highest amount of SDS was eluted in the third fraction and it was eluted completely with 2 ml methanol. From these findings, it is proposed that first fraction of 1 ml was discard after elution with methanol, and subsequently the second fraction of 1 ml was collected for the analysis of SDS in water samples.

Table 3.16 Effect of eluent volume on desorption of SDS

No. of fraction	Eluent volume (ml)	Peak area (mAU*s)
1	0.5	n.d.
2	1.0	n.d.
3	1.5	72.20
4	2.0	5.23
5	2.5	n.d.
6	3.0	n.d.
7	3.5	n.d.
8	4.0	n.d.

n.d., not detected.

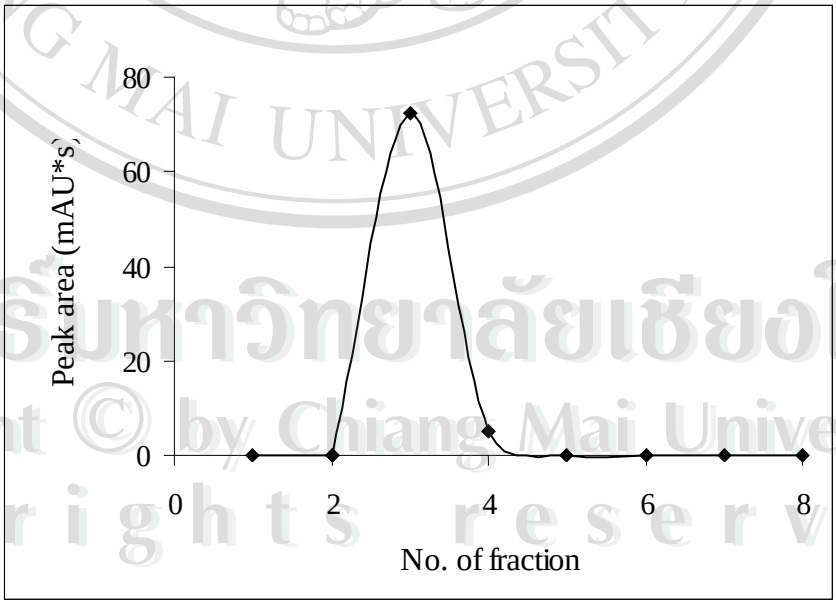

Fig. 3.34 Elution profile of SDS obtained using C_{18} cartridge.

3.3.4 Analysis of SDS in water samples

Several natural water and wastewater extracts were analysed in order to demonstrate that the method proposed in this work is suitable for the analysis of SDS in real samples. The chromatograms (**Fig. 3.35**) showed the presence of SDS peak in natural water and wastewater samples. However, the early eluting peak (negative peak) originating from sample impurities affected the peak shape of SDS. So peak area of SDS was difficult to measure for the determination of SDS in these samples. It was observed that it could be used for identification of SDS in real water samples.

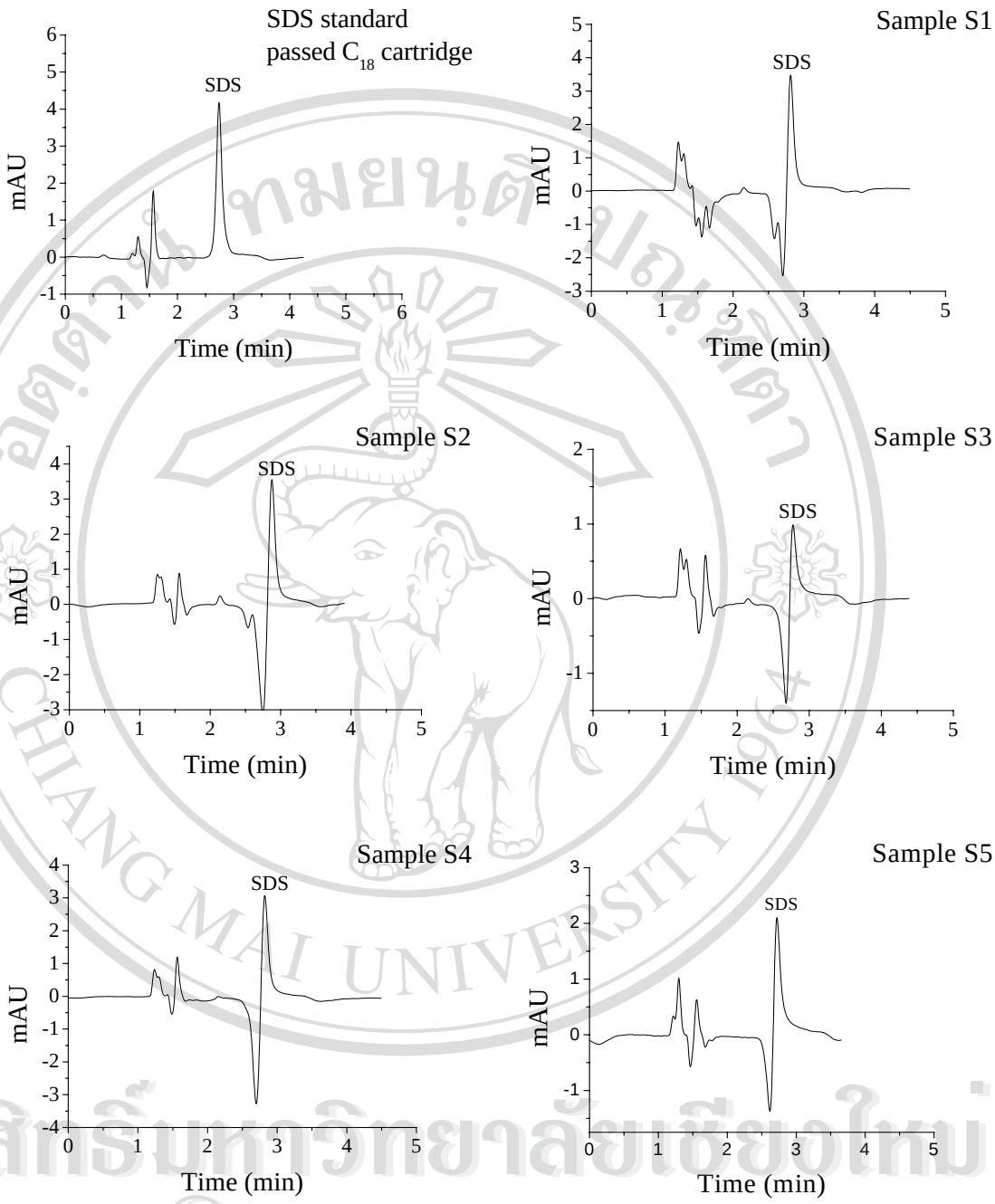

Fig. 3.35 Chromatograms of SDS in water extracts under the proposed condition.

S1, natural water in Mae-Ping river, Chiang Mai; S2, natural water from irrigation canal, Chiang Mai; S3, natural water in Ang-Kaew reservoir, Chiang Mai University, S4, wastewater in Mae-Kha canal, Chiang Mai; S5, drainage water from student dormitory Chiang Mai University.