CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and Chemicals

2.1.1 Apparatus

1) High performance liquid chromatographic system (HP1100) manufactured by Agilent Technologies, U.S.A., consists of quaternary pump, vacuum degasser, Rheodyn mannual injector valve (20 μl loop), UV-VIS detector and data processing system (HP chemstation).

2) High performance liquid chromatography - mass spectrometric system manufactured by Agilent Technologies, U.S.A., consists of binary pump, vacuum degasser, auto injector, mass spectrometer detector and data processing system (HP chemstation).

3) UV-VIS spectrophotometer (Lamda-25), Perkin-Elmer, U.S.A.

4) Analytical column, a Zorbax Eclipse XDB C_8 column, 150 x 4.6 mm I. D., 5 μm, Agilent, U.S.A.

5) Glass syringe, 100 μl, Hamilton, U.S.A.

6) Filter unit, Millipore, U.S.A.

7) Filter membrane, 0.45 μm, Sartorius, Germany

8) Ultrasonicator model 8891, Cole-Parmer, U.S.A.

9) Vacuum pump, Gast, U.S.A.

10) C_{18} cartridge, Sep-Pak Cartridge, Waters, U.S.A.

11) pH meter model pH 744, Metrohm, U.S.A.

12) Analytical balance, Mettler Toledo, Switzerland

2.1.2 Chemicals

The chemicals used in this work with their purity grade and suppliers are listed in **Table 2.1**.

Table 2.1 List of chemicals used, their purity grade and suppliers

Chemical	Purity grade	Supplier
Linear alkylbenzene sulfonate (LAS), sodium salt	Commercial	Henkel, Germany
Triton X-100	99%	BDH, England
Sodium dodecyl sulfate (SDS)	98%	BDH, England
Methanol	HPLC	BDH, England
Sodium chloride	A.R.	Carlo Erba, Spain
Sodium acetate	A.R.	Carlo Erba, Spain
Ammonium acetate	Puriss	Fluka, Switzerland
Lidocaine	A.R.	Sigma, U.S.A.
Phosphoric acid	A.R.	J.T. Baker, U.S.A.

2.2 Preparation of the Solutions

2.2.1 Preparation of surfactant standard stock solutions

Each stock solution of surfactant standard (1000 ppm) was prepared by dissolving 100 mg of each surfactant, i.e. LAS, Triton X-100 and SDS in 100 ml of Milli-Q water. Before use, the stock solution was further diluted to 100 ppm and desired concentrations.

2.2.2 Preparation of mobile phase

2.2.2.1 Analysis of LAS and Triton X-100

The investigation for suitable mobile phase was initiated using four types of binary solvent systems where methanol was mixed with water or three common salt solutions, i.e. sodium chloride, sodium acetate or ammonium acetate. Common salt solutions were prepared by dissolving the appropriate weight of each salt in Milli-Q water.

For example, the preparation of 10 mM sodium chloride was done by dissolving 0.2922 g of sodium chloride in 500 ml of Milli-Q water. In case of 10 mM sodium acetate and 10 mM ammonium acetate solutions were prepared similar to the sodium chloride solution but the weights of sodium acetate and ammonium acetate used were 0.6804 g and 0.3854 g, respectively.

All mobile phase solutions were filtered using a filter unit with 0.45 μm filter membrane and a vacuum pump. Finally, the prepared solutions were degassed for 20 minutes using ultrasonicator.

2.2.2.2 Analysis of SDS

The mobile phase solution was prepared by mixing methanol, water and lidocaine solution and then adjusted pH with 5% phosphoric acid. The lidocaine solution (1000 ppm) was done by dissolving 50 mg of lidocaine in 50 ml methanol.

The preparation of 1000 ml of a mobile phase solution consisting of 40% methanol in water containing 5 ppm lidocaine at pH 3.4 was done by mixing 5 ml of 1000 ppm lidocaine and 400 ml methanol. The solution was diluted with Milli-Q water until the volume near the mark. Then the solution was adjusted to pH 3.4 with 5% phosphoric acid and the solution was finally adjusted volume to 1000 ml with Milli-Q water. The mobile phase solution was filtered using 0.45 μm filter membrane and degassed for 20 minutes using ultrasonicator.

2.3 Liquid Chromatographic Analysis

The determination of some anionic and nonionic surfactants in various water samples by high performance liquid chromatography has been investigated. The experimental procedures for this technique are shown in **Fig. 2.1**.

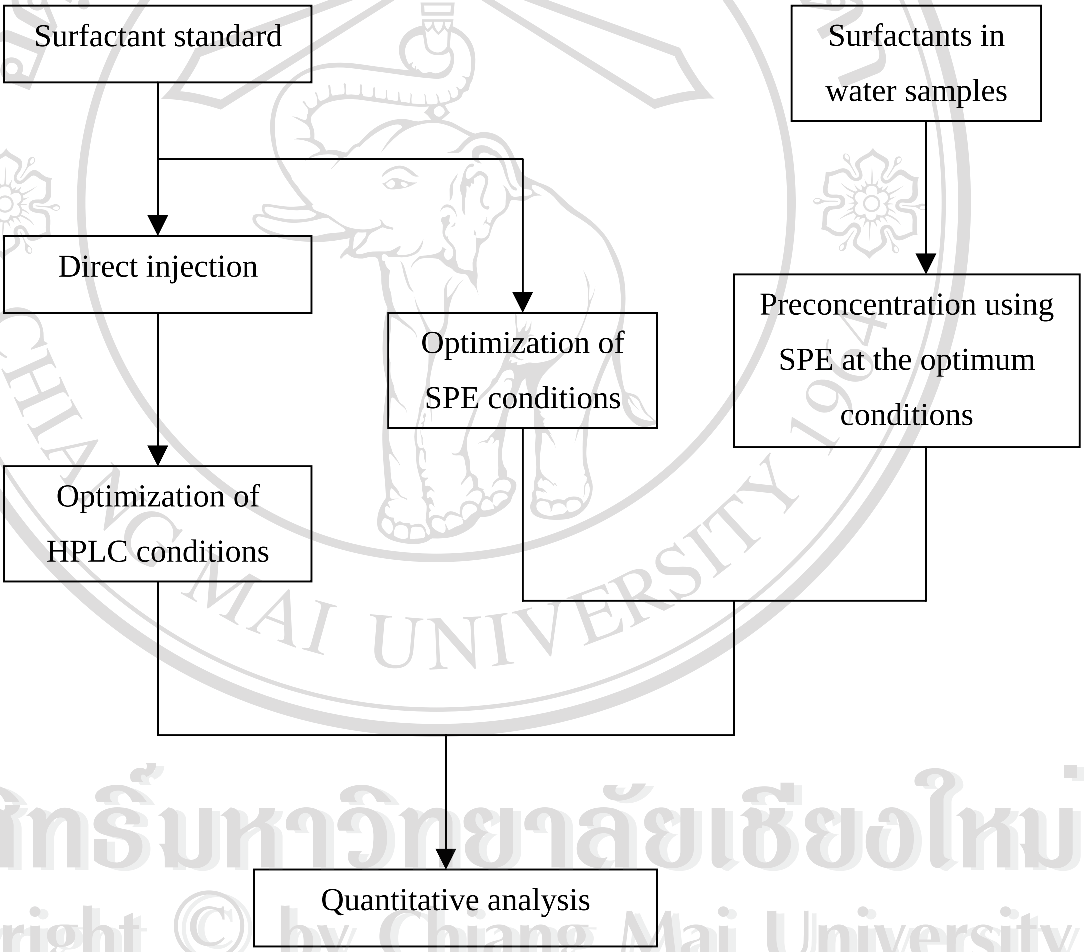

Fig. 2.1 Summary of experimental procedures for the determination of surfactants by HPLC.

In this work, the experiment was divided into three parts. Firstly, the separation of LAS homologues was investigated using various binary solvent systems in RP-HPLC. Secondly, the conditions of HPLC and SPE were optimized for the simultaneous determination of LAS and Triton X-100. Finally, the HPLC and SPE conditions were optimized for the analysis of SDS. Moreover, precision, detection limit and accuracy of these proposed methods were carried out under the optimum conditions.

2.3.1 Separation of LAS compounds

Optimum separation of LAS homologues is achieved by adjusting the compositions of mobile phase, types and the concentrations of common salts (sodium chloride, sodium acetate and ammonium acetate). A 20 μl of 3 ppm LAS standard was injected onto the column (XDB C_8, 150 x 4.6 mm I.D., 5 μm) at the constant flow rate of mobile phase (1.0 ml min^{-1}). The confirmation of these compounds was investigated using mass spectrometry.

2.3.1.1 Optimization of HPLC conditions

a) Detection wavelength

The absorption spectrum of 5 ppm LAS standard solution was examined in the wavelength between 200 to 350 nm using UV-VIS spectrophotometer.

b) Mobile phase composition

The binary solvent system investigated in this work was the mixture of methanol and water. Mobile phase compositions in the range of 70-80% methanol in water were carried out. A 20 μl of 3 ppm LAS standard was injected onto the column at the flow rate of 1.0 ml min^{-1} and the detection wavelength of 224 nm.

c) Type and concentration of salt

Three types of common salt, i. e. sodium chloride, sodium acetate and ammonium acetate were employed at the concentration ranging from 1 to 10 mM adding into the mixture of methanol/water (80/20, v/v). A 20 μl of 3 ppm LAS standard was injected onto the column at the flow rate of 1.0 ml min^{-1} and the detection wavelength of 224 nm.

2.3.1.2 Confirmation of LAS compounds

A 40 μl of 4 ppm LAS standard was injected onto the column at the constant flow rate of mobile phase (0.5 ml min^{-1}) and detected using negative-ion electrospray (ES) – mass spectrometry (MS). The mobile phase containing ammonium acetate was employed. The MS conditions used were fragmentor voltage 160 volts, nebulizer pressure 20 psi, drying gas flow rate 10 l min^{-1}, drying gas temperature 350 °C and capillary voltage 3,500 volts.

2.3.2 Analysis of LAS and Triton X-100

2.3.2.1 Optimization of HPLC conditions

a) Detection wavelength

Triton X-100 standard solution (10 ppm) was scanned for the absorption in the wavelength range of 200-350 nm using UV-VIS spectrophotometer.

b) Mobile phase composition

The mobile phase used was the mixture of methanol and water containing ammonium acetate. The amounts of methanol (75% and 80%) and the concentrations of ammonium acetate (1-5 mM) were optimized. A 20 μl of the standard mixture containing 3 ppm LAS and 4 ppm Triton X-100 was injected onto the column at the flow rate of 1.0 ml min^{-1} and the detection wavelength of 224 nm.

c) Mobile phase flow rate

The optimum mobile phase flow rate was carried out by injecting a 20 μl of the standard mixture containing 3 ppm LAS and 4 ppm Triton X-100 onto the column using the mixture of 75% methanol in water containing 2 mM ammonium acetate as mobile phase at the flow rates of 0.8-1.2 ml min^{-1} and the detection wavelength of 224 nm.

2.3.2.2 Precision

The standard mixture containing 3 ppm LAS and 4 ppm Triton X-100 was employed. The repeatability was investigated using eleven injections of the standard mixture in the same day and the reproducibility was determined in the different days for seven injections under the optimum conditions.

2.3.2.3 Detection limit

The standard solutions were prepared in the range of 21-62 ppb for C_{10} LAS, 17-86 ppb for C_{11} LAS, 18-89 ppb for C_{12} LAS, 39-117 ppb for C_{13} LAS and 100-300 ppb for Triton X-100. A 20 μl of each standard mixture in **Table 2.2** was injected onto the column under the optimum conditions.

Table 2.2 Concentration of LAS and Triton X-100 in standard mixtures

Solutions	Concentration (ppb)				
	C_{10} LAS	C_{11} LAS	C_{12} LAS	C_{13} LAS	Triton X-100
1	21	17	18	39	100
2	31	34	36	59	150
3	42	51	54	78	200
4	52	69	72	98	250
5	62	86	89	117	300

2.3.2.4 Elution profile of LAS and Triton X-100 using C_{18} sorbent

SPE cartridge packed with 500 mg of C_{18} sorbent was used. The cartridge was first conditioned by passing 7 ml methanol and 7 ml water before use. Methanol was used as eluting solvent. The 0.5 ml of standard mixture (2 ppm LAS and 4 ppm Triton X-100) was directly spiked to conditioned C_{18} cartridge. Then the cartridge was rinsed with 6 ml of the mixture of 30% methanol in water. Finally, the cartridge was eluted using methanol. The eluate was collected as a series of 0.5 ml fraction and adjusted volume to 1 ml with Milli-Q water before injecting to the optimum HPLC system.

2.3.2.5 Determination of LAS and Triton X-100 in water samples

a) Quantitative analysis

Water samples (100 ml) were filtered through Whatman filter paper and then they were subjected to purification and preconcentration on the C_{18} cartridge under proposed SPE condition. The extracts were analyzed using the optimum HPLC system. In addition, spiked water samples consisting of 1 μg LAS and 2 μg Triton X-100 were analyzed. The SPE procedures for determining LAS compounds and Triton X-100 in water samples are summarized in **Fig. 2.2**.

b) Identification of LAS compounds in water sample

The negative-ion electrospray (ES) – mass spectrometry (MS) was used for the confirmation of LAS compounds in water samples. The extracted water samples were injected onto the column using 80% methanol in water containing 1.5 mM ammonium acetate at the flow rate of 0.5 ml min^{-1}. The MS conditions used were fragmentor voltage 160 volts, nebulizer pressure 20 psi, drying gas flow rate 10 l min^{-1}, drying gas temperature 350 °C and capillary voltage 3,500 volts.

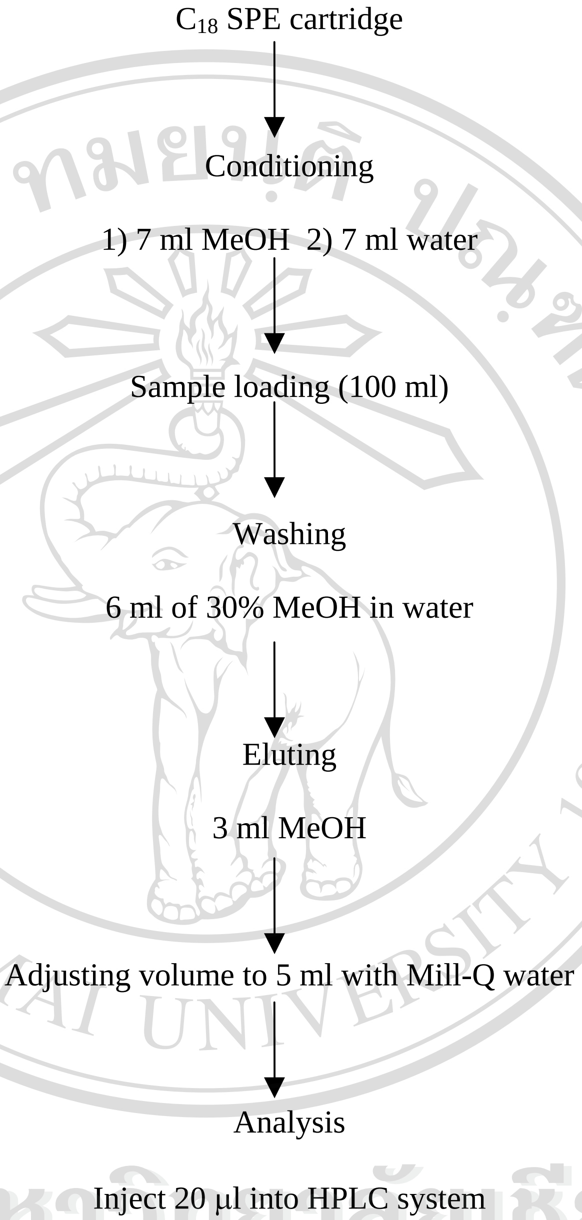

Fig. 2.2 Summary of SPE procedures for the determination of LAS and Triton X-100.

2.3.3 Analysis of SDS

2.3.3.1 Optimization of HPLC conditions

a) Detection wavelength

A 20 µl of 10 ppm SDS standard solution was injected into the chromatographic system using 40% methanol in water containing 1 ppm lidocaine at pH 3.0 with a flow rate 1.0 ml min^{-1}. The detection wavelengths in the range of 230-250 nm were investigated.

b) Mobile phase composition

Mobile phase used was the mixture of methanol and water containing 1 ppm lidocaine at pH 3.0. Mobile phase compositions in the range of 30-50% methanol in water were investigated. A 20 µl of 10 ppm SDS standard was injected onto the column at the flow rate of 1.0 ml min^{-1} and the detection wavelength of 230 nm.

c) pH of the mobile phase

The pH of 40% methanol in water containing 1 ppm lidocaine was varied in the range of 3.0-4.0. A 20 µl of 10 ppm SDS standard was injected onto the column at the flow rate of 1.0 ml min^{-1} and and the detection wavelength of 230 nm.

d) Concentrations of lidocaine

The concentrations of lidocaine in 40% methanol in water at pH 3.4 were investigated in ranging 0.1–6.5 ppm. A 20 µl of 10 ppm SDS standard was injected onto the column at the flow rate of 1.0 ml min^{-1} and the detection wavelength of 230 nm.

2.3.3.2 Precision

The precision of this proposed method was investigated in term of repeatability and reproducibility same as Section 2.3.2.2. by injecting 10 ppm SDS standard solution.

2.3.3.3 Detection limit

The SDS standard solutions were prepared in the range of 1-3 ppm. A 20 μl of standard solution was injected onto the column under the optimum conditions.

2.3.3.4 Elution profile of SDS using C_{18} sorbent

SPE cartridge packed with 500 mg of C_{18} sorbent was used. The cartridge was conditioned by passing 7 ml methanol and 7 ml water before use. The 0.5 ml of 20 ppm SDS standard was directly spiked to conditioned C_{18} cartridge. The cartridge was rinsed with 6 ml of the mixture of 30% methanol in water. Then the cartridge was eluted using 2 ml of methanol. A series of 0.5 ml fraction of the eluate was collected and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 1 ml of HPLC mobile phase before injecting onto column under the optimum HPLC condition.

2.3.3.5 Analysis of SDS in water samples

Water samples were filtered through Whatman filter paper and then they were purified and preconcentrated on the C_{18} cartridge. The extracts were analyzed under the optimum HPLC condition.