CHAPTER 2
EXPERIMENTAL

2.1 Apparatus and Chemicals

2.1.1 Apparatus

1) Gas chromatograph, Agilent 6890 Series GC system, manufactured by Hewlett Packard, U.S.A., consisting of

a) Flame ionization detector (FID)

b) Data processing system, Dell OptiPlex GX 1

c) Capillary columns :

HP-FFAP, 25 m x 0.32 mm I.D., 0.50 μm film thickness, Hewlett Packard, U.S.A.

INNOWAX, 30 m x 0.32 mm I.D., 0.50 μm film thickness, Hewlett Packard, U.S.A.

2) Gas chromatograph-mass Spectrometry, Hewlett Packard 5973 Mass Selective Detector manufactured by Hewlett Packard, U.S.A., consisting of

a) Gas chromatograph, Agilent 6890 Series GC system manufactured by Hewlett Packard

b) Data processing system, Hewlett Packard, U.S.A.

c) INNOWAX, 30 m x 0.32 mm I.D., 0.50 μm film thickness, Hewlett Packard, U.S.A.

3) Solid phase microextraction fiber holder, manual sampling manufactured by Supelco, U.S.A.

4) Solid phase microextraction fiber, 100 μm Polydimethylsiloxane manufactured by Supelco, U.S.A.

5) Analytical Balance, Satorious Basic BA 210s ,manufactured by Sartorious AG, Germany

6) Cooling device, NESLAB, manufactured by NESLAB instruments, Inc., U.S.A.

7) Magnetic stirrer, Thermolyne, U.S.A.

8) Autopipette, manufactured by Brand Tech Scientific Inc., U.S.A.
9) Hand crimper, 20 mm cap, manufactured by Supelco, U.S.A.
10) Magnetic sterring bar 10 x 3 mm, Merck, U.S.A.
11) Headspace vial 22 ml, Hewlett Packard, U.S.A.
12) Septa (PTFE faced silicone) 22 mm, Supelco, U.S.A.

2.1.2 **Chemicals**

1) Methanol, HPLC Grade, Carlo Erba , Italy
2) Benzene, > 99.5 % , Merck, Germany
3) Toluene, > 99.0 % BDH, England
4) Ethylbenzene, > 98.0 %, Fluka, Switzerland
5) p-Xylene, > 99.5 % , Fluka, Switzerland
6) m-Xylene, > 99.5 % , Fluka, Switzerland
7) o-Xylene, > 99.0 % , Merck, Germany
8) Sodium chloride, AR grade, Merck , Germany
9) Ultrapure water (Milli-Q system, Millipore, Beldford, MA, U.S.A.)
10) Helium gas, 99.99% (HP grade), TIG, Thailand
11) Nitrogen gas, 99.999% (UHP grade), TIG, Thailand
12) Air, Air-Zero grade, TIG, Thailand
13) Hydrogen, 99.99% (HP grade)

2.2 Preparation of Standard Solutions of BTEX Compounds

2.2.1 Preparation of individual of BTEX stock standard solutions

Each of benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene stock standard solution with concentration of 100 mg/10 ml was prepared. For each compound, methanol was used as a solvent. Firstly, a 10 ml volumetric flask with cap was accurately weighed using an analytical balance and the calculated volume of the single standard was pipetted into the volumetric flask. The volumetric flask was closed with a cap and the volumetric flask was accurately weighed again. Without any delay, methanol was transferred into a 10 ml volumetric flask to the 10 ml mark.

The calculated volume is the volume of 100 mg of each standard substance. For example, in the preparation of benzene stock solution, the calculated volume of benzene could be obtained in the following manner [63].

Given the density of benzene = 0.866 g/ml

% purity = 99.5 %

Therefore 100 mg of benzene = $\frac{0.1}{0.866} \times \frac{100}{99.5}$

= 114 µl

2.2.2 Preparation of primary mixed BTEX standard solutions

The BTEX mixed primary standard solution with concentration of 2,000 g/ml benzene and 1,000 µg/ml of toluene, ethylbenzene, p-xylene, m-xylene and o-xylene in methanol was prepared by transferring 2 ml of 10 mg/ml benzene solution and 1 ml of 10 mg/ml of toluene, ethylbenzene, p-xylene, m-xylene and o- xylene standard solution to a 10 ml volumetric flask and the volume was adjusted to the 10 ml mark with methanol.

2.2.3 Preparation of mixed BTEX working standard solutions

Two BTEX mixed working standard solutions in methanol, First dilution and Second dilution solution were prepared to obtained different but appropriate concentrations of the BTEX compounds as listed in Table 2.1.

Table 2.1 Concentrations of working standard solutions of BTEX compounds.

BTEX compound	*Concentration of working standard solutions of BTEX compounds (μg/ml)	
	First dilution	**Second dilution**
Benzene	200	20
Toluene	100	10
Ethylbenzene	100	10
p-Xylene	100	10
m-Xylene	100	10
o-Xylene	100	10

* **First dilution solution** was prepare from primary mixed BTEX standard solution by means of ten-fold dilution in a 10-ml volumetric flask. **Second dilution solution** was prepared from First dilution by means of ten-fold dilution in a 10-ml volumetric flask.

In this work, the primary and working standard solutions of BTEX in methnol were prepared for use in spiking Milli-Q water and water samples to yield aqueous solutions of BTEX in water.

2.2.4 Preparation of aqueous BTEX standard solutions

All aqueous BTEX standard solutions were prepared by spiking an appropriate amount of the primary or working standard into volumetric flask containing Milli-Q water and the volume was made to the mark with blank water. The content of methanol in the spiked aqueous solutions was always less than 1 % by volume. Aqueous solutions were prepared daily.

2.2.4.1 Aqueous BTEX standard solutions for optimization of solid- phase microextraction

Four sets of the aqueous BTEX standard solutions were prepared by dilution in Milli-Q water of the working standard solutions to give the concentrations presented in Table 2.2.

Table 2.2 The concentrations of aqueous solutions for optimization of solid-phase microextraction

Component	Aqueous standard solution			
	Solution A[a] (μg/L)	Solution B[a] (μg/L)	Solution C[b] (μg/L)	Solution D[b] (μg/L)
Benzene	4	40	200	400
Toluene	2	20	100	200
Ethylbenzene	2	20	100	200
p-Xylene	2	20	100	200
m-Xylene	2	20	100	200
o-Xylene	2	20	100	200

[a] Aqueous solutions were prepared by dilution of the second dilution solution of working standard solution with the Milli-Q water.

[b] Aqueous solutions were prepared by dilution of the first dilution solution of working standard solution with Milli-Q water.

2.2.4.2 Aqueous BTEX standard solutions for constructing the calibration curves

Five sets of the aqueous BTEX standard solutions used in construction the calibration curve consisting of aqueous solutions set A , B , C , D and E :

- Aqueous BTEX standard solutions set A

These aqueous solutions were used in constructing the calibration curve for determination of the linearity range for the BTEX compound analysis in water of the

optimum headspace SPME conditions which were prepared by dilution of the primary and working standard solutions in Milli-Q water to give the concentrations listed in Table 2.3.

Table 2.3 The concentrations of BTEX compounds prepared in aqueous BTEX standard solutions set A for construction of calibration curve.

Component	BTEX concentration (μg/L)				
	Standard[a] number 1	Standard[a] number 2	Standard[a] number 3	Standard[b] number 4	Standard[c] number 5
Benzene	2	20	40	200	2,000
Toluene	1	10	20	100	1,000
Ethylbenzene	1	10	20	100	1,000
p-Xylene	1	10	20	100	1,000
m-Xylene	1	10	20	100	1,000
o-Xylene	1	10	20	100	1,000

[a] Aqueous solutions were prepared by dilution of the second dilution solution of working standard solution with the Milli-Q water.

[b] Aqueous solutions were prepared by dilution of the first dilution solution of working standard solution with the Milli-Q water.

[c] Aqueous solution were prepared by dilution of the primary standard solution with the Milli-Q water.

- Aquoues BTEX standard solutions set B

These aqueous solutions were used in constructing the calibration curve for the recovery test at the low range BTEX concentration of the optimum headspace solid-phase microextraction conditions for BTEX compounds analysis in water which were prepared by dilution of the second dilution solution of working standard solutions in Milli-Q water to give the concentrations listed in Table 2.4

Table 2.4 The concentrations of BTEX compounds prepared in aqueous BTEX standard solutions set B for construction of calibration curve.

Component	BTEX concentration (μg/L)				
	Standard number 1	Standard number 2	Standard number 3	Standard number 4	Standard number 5
Benzene	2	4	6	8	10
Toluene	1	2	3	4	5
Ethylbenzene	1	2	3	4	5
p-Xylene	1	2	3	4	5
m-Xylene	1	2	3	4	5
o-Xylene	1	2	3	4	5

- Aqueous BTEX standard solutions set C

These aqueous solutions used in constructing the calibration curve for the recovery test at medium and high ranges BTEX concentration of the optimum headspace solid-phase microextraction conditions for BTEX compounds analysis in water which were prepared by dilution of the primary and working standard solutions in Milli-Q water to give the concentrations presented in Table 2.5.

Table 2.5 The concentrations of BTEX compounds prepared in aqueous solutions set C for construction of calibration curve.

Component	BTEX concentration (μg/L)				
	Standard[a] Number 1	Standard[a] number 2	Standard[a] number 3	Standard[b] number 4	Standard[c] number 5
Benzene	4	10	20	200	2,000
Toluene	2	5	10	100	1,000
Ethylbenzene	2	5	10	100	1,000
p-Xylene	2	5	10	100	1,000
m-Xylene	2	5	10	100	1,000
o-Xylene	2	5	10	100	1,000

[a] Aqueous solutions were prepared by dilution of the second dilution solution of working standard solution with the Milli-Q water.

[b] Aqueous solutions were prepared by dilution of the first dilution solution of working standard solution with the Milli-Q water.

[c] Aqueous solution were prepared by dilution of the primary standard solution with the Milli-Q water

- Aqueous BTEX standard solutions set D

These aqueous solution were used in constructing the calibration curve for determination of BTEX compounds in real water samples, obtained from a motorbike service garage. The optimum headspace solid-phase microextraction conditions obtained were based on dilution of the second of working standard solution in Milli-Q water to give the concentrations listed in table 2.6.

Table 2.6 : The concentrations of BTEX compounds prepared in aqueous standard solutions set D for construction of calibration curve.

Component	BTEX concentration (μg/L)				
	Standard Number 1	Standard number 2	Standard number 3	Standard number 4	Standard number 5
Benzene	2	4	8	12	16
Toluene	1	2	4	6	8
Ethylbenzene	1	2	4	6	8
p-Xylene	1	2	4	6	8
m-Xylene	1	2	4	6	8
o-Xylene	1	2	4	6	8

- Aqueous standard solutions set E

These aqueous solutions used in constructing the calibration curve for determination of toluene in real water samples. Industrial wastewater sample analyzed with the optimum headspace solid-phase microextraction conditions were prepared by first, dilution of 100 μl of 10 mg/ml toluene stock standard solution with 10 ml methanol to give the concentrations 100 μg/ml toluene, and then diluted to aqueous standard solution set E with Milli-Q water to give the concentrations listed in Table 2.7.

Table 2.7 The concentrations of toluene prepared in aqueous standard solutions set E for construction of calibration curve.

Standard number	Toluene concentration (μg/L)
1	50
2	100
3	150
4	200
5	300

2.3 Gas Chromatographic Conditions

In this research, gas chromatographic conditions for separation of some BTEX compounds were investigated. Gas chromatographic investigations were carried out in order to obtain adequate separation of peaks on the chromatogram with both reasonably shortest analysis time and good resolution possible. The optimum conditions obtained were used for determination of some BTEX compounds in water sample.

Gas chromatographic separation was achieved on a gas chromatograph equipped with a flame ionization detector. To confirm the studied compounds and results obtained for the real samples, GC-MS analyses were performed using a quadupole mass spectrometric detector. All injections with the SPME unit were performed manually by using a SPME fiber holder. Both the optimum conditions of GC-FID and GC-MS employed are shown in Table 2.8 and Table 2.9, respectively.

Table 2.8 Conditions of GC-FID employed

Operation	Conditions
1. Column	HP-FFAP , 25 m x 0.32 mm I.D., 0.32 μm film thickness
2. Temperature program	65 °C (12 min) – 150 °C (13 °C/min) 150 °C (1.46 min)
3. Injector temperature	200 °C
4. Detector temperature	220 °C
5. Injection	Splitless mode with SPME insert
6. Carrier gas	Helium at 1.0 ml/min
7. Detector makeup	Nitrogen at 45 ml/min
8. Hydrogen	40 ml/min
9. Air	450 ml/min

Table 2.9 Conditions of GC-MS EI mode employed

Operation	Conditions
1. Column	INNOWAX, 30 m x 0.32 mm I.D., 0.32 μm film thickness
2. Temperature program	65 °C (8 min) – 150 °C (13 °C/min) 150 °C (1.46 min)
3. Injector temperature	200 °C
4. Injection	Splitless mode with SPME insert
5. Carrier gas	Helium at 1.5 ml/min
6. Transfer-line temperature	200 °C
7. Ion source temperature	230 °C
8. Mass range	40 - 250 amu
9. Ionization mode	EI , 70 eV
10. MS quadrupole	150 °C

2.4 Optimization of Solid-Phase Microextraction

2.4.1 SPME conditioning

In this study, a manual SPME holder was used to perform the experiments with a fused silica fiber of 10 mm in length, 100 μm in diameter, and with 100 μm thickness of polydimethylsiloxane chosen to extract the BTEX components of the water samples. In the initial work, a new PDMS microextraction fiber must be thermally conditioned prior to its first adsorption at 250 °C in an injection port of GC under helium for 1 h to reduce bleeding before use. This can be achieved by inserting the SPME syringe needle into a split/splitless injector while the purge is open. After conditioning, a fiber blank was run to ensure that no contaminant was in the fiber coating prior to exposure of the fiber to the given sample. The conditioned fibers were used immediately or protected from contamination by inserting the SPME syringe needle into a GC septum injection port before use. Between uses, fibers were

kept sealed from ambient air by piecing the tip of SPME needle into a small pierce of septum to prevent accidental contamination. Prior to each use, all used fibers were preconditioned by thermal desorption at 220 °C for 20 min in injection port of GC. If any carry over was observed by GC/FID, the 20 min thermal preconditioning was repeated.

2.4.2 Preparation for analysis of BTEX by SPME sampling technique

First, 10-ml of aqueous test solution was transferred to a 22-ml glass vial with a pipette of sufficient capacity to deliver the entire solution in one step. Then, a magnetic stirring bar was to introduced. The vial was sealed rapidly with a PTFE septum and aluminum cap.

2.4.3 Preparation for analysis of BTEX by HSSPME sampling technique

Sample vials were prepared by first weighing 3 g of sodium chloride into an empty 22-ml glass vial. Then 10-ml of aqueous test solution was transferred with a pipette of sufficient capacity to deliver the entire solution in one step and a magnetic stirring bar was placed into vial which was sealed and crimped quickly.

2.4.4 SPME sampling and analysis procedure

After addition of the aliquot and a stirrer bar to the vial, SPME extractions were performed on 22-ml vial in direct SPME or headspace SPME sampling. All sampling was conducted at ambient temperature (24-25 °C) while the aqueous phase was under constant magnetic stirring of 70% of maximum speed. The sampling time for both direct SPME and headspace SPME was designated as extraction time, based on the optimization of SPME extraction. The experiments were carried out according to the scheme in Figure 2.1.

Place the vial on a magnetic stirrer
and mount it in a clamp
⇓
set the stirrer at 70% of maximum speed
⇓
pierce the septum of the vial with needle of SPME device
and fasten the device with a clamp
⇓
expose the fiber by depressing the plunger
and lock it in the bottom position by turning it clockwise.
⇓
start the timer
⇓
after exactly time of the designed extraction time
withdraw the fiber back into the needle
and pull the device out of the vial
⇓
introduce the needle of the SPME device to the injector of GC
⇓
start the analysis by depressing the plunger
and locking it in the " lowered " position
⇓
after exactly 2 min
withdraw the fiber back into the needle
and pull the needle out of the injector
⇓
subsequent analyze by either GC-FID or GC-MS

Figure 2.1 Scheme of SPME sampling and analysis procedure

2.4.5 Comparison between SPME and HSSPME technique

Two sample techniques were investigated. One involved immersing the fiber in aqueous phases (SPME) and, in the other, the fiber was suspended in the headspace above the water (HSSPME). 10-ml spiked water, prepared by the same procedure as solution C of aqueous BTEX standard solution preparation for the studying the optimization of SPME, was used in both cases. An adsorption time of 10 min and aqueous solution was under constant stirring at 70% of maximum speed was used in this study. Once sampling was complete, the fiber was desorbed for 2 min at 200 °C was enough for quantitative desorption of all the analyses studied and no carry over was observed. Triplicate analyses were performed for all experiments. Comparison results obtained are summarized in Table 3.3 and Figure 3.11. Figures 3.1, 3.2 and show a representative chromatograms obtained by SPME and HSSPME technique.

2.4.6 Determination of the optimal conditions for HSSPME

In order to develop an HSSPME technique for the analysis of BTEX compounds in water, it is necessary to optimize several parameters. In the study, all the optimal conditions for headspace sampling, including stirring speed, ionic strength influence, extraction time and temperature were investigated. The investigation was carried out as follows.

2.4.6.1 Investigation of stirring speed

In this work, conventional magnetic stirring bar was used for agitation in the SPME experiments. The effect of stirring speed with aqueous solution C of aqueous BTEX standard solutions for studying the optimization of SPME was obtained by monitoring the peak areas as a function of stirring speed which was varied between 0% and 80% of maximum speed. For this study, salt was not added to an 10 ml aqueous solution C before extraction. HSSPME was performed with extraction time at ambient temperature for 10 min and thermally desorbed at 200 °C for 2 min, then subsequent analyzed by GC-FID. All procedures were carried out in duplicate and the results are illustrated in Table 3.4 and Figure 3.12.

2.4.6.2 Investigation of the effect of added salt

The effect of the addition of salt to the aqueous solution C was also studied. For this study, an 10 ml of aqueous solutions was saturated with 3 g NaCl before extraction. BTEX determination was tested by saturating an aqueous solution with salt and comparing the results with those obtained without salt addition. HSSPME was performed as in the previous step and the aqueous phase was under constant magnetic stirring of 70% of maximum speed. All procedures were carried out in triplicate and comparison results obtained are illustrated in Table 3.5 and Figure 3.13. Figure 3.2 and Figure 3.3 shows a representative the chromatograms obtained by both HSSPME technique with and without salt addition.

2.4.6.3 Determination of adsorption-time profile

The BTEX adsorption-time profile on the PDMS fiber was studied by monitoring the area counts as a function of exposure time from 3 to 20 min. A 10-ml of aqueous solution C was used in this study and it was the same as in the previous study and the solution was saturated with 3 g NaCl before extraction. All the extractions were carried out at ambient temperature (24-25 °C) and aqueous solution was under constant stirring at 70% of maximum speed. After the adsorption process, the analytes were thermally desorbed at 200 °C for 2 min, then subsequent analyzed by GC-FID. All procedures were carried out in duplicate and the results obtained are illustrated in Table 3.6 and Figure 3.14.

In addition, the BTEX adsorption-time profile into the different three PDMS fibers were also investigated by monitoring the peak areas as function of both time and the different three PDMS fibers by varying the exposure time between 3 and 12 min . Three sets of aqueous standard solutions consisting of solution A, B and D were used in this study and their preparation is presented in Table 2.2, and each of a 10 ml aqueous solution was also saturated with 3 g NaCl before extraction. All the extractions and analysis of HSSPME were performed as in the previous step. The adsorption-time profile for six chemicals of BTEX on the different three PDMS fibers are presented in Figures 3.15 – 3.20.

2.4.6.4 Determination of extraction-temperature profile

The influence of the temperature on the analyte adsorption was investigated in the range 2-40 °C with the aqueous solution C which were also saturated with 3 g NaCl before extraction. HSSPME was performed with extraction time for 10 min while the aqueous phase was under constant magnetic stirring at 70% of maximum speed and thermally desorbed at 200 °C for 2 min, then subsequent analyzed by GC-FID. All procedures were carried out in duplicate and the results obtained are illustrated in Table 3.7 and Figure 3.21.

2.5 Detection Limits, Linearity and Precision Study

After establishing the optimized conditions of solid-phase microextraction, the detection limits, linearity and precision were investigated by extracting spiked aqueous BTEX standard solution set A as described in the Section 2.2.4.2.

2.5.1 Detection limits

In this study, the lowest detection limits were obtained with flame ionization detection. The detection limits for BTEX compounds in ultrapure water attained at optimum conditions were performed by comparing measured signals from peak height of the GC-FID chromatogram of BTEX with known low concentration of analyte of 1 µg/L for benzene and 0.5 µg/L for the other five analytes , with these of blank samples. The acceptance criteria was a signal/noise ratio (S/N) of a minimum 3:1. All procedures were carried out in replicates of three. The detection limits of the employed method are summarized in table 3.9.

2.5.2 Linearity

To evaluate the linearity of the optimized method, a calibration study was performed by preparing spiked aqueous BTEX standard solution set A as described in the Section 2.2.4.2 to give five concentration levels covering the range of 2 to 2000 µg/L for benzene and 1 to 1000 µg/L for the other five analytes. At each concentration level, at least triplicate analytes were made. All the compounds studied were characterised by regression coefficients of the calibration curve not below 0.9999 and relevant plots are shown in Figures 3.22- 3.27.

2.5.3. Precision

The precision of the optimized method for the determination of BTEX compounds in water was determined by six extractions with spiked ultapure water of 4 µg/L for benzene and 2 µg/L of the other five analytes, The results of detection limits, linearity and precision for the determination of BTEX compounds in ultrapure water are summarized in Table 3.10.

2.6 Recovery assay

Under the optimal conditions of the research work, efficiency of the method was investigated with recovery test by spiking BTEX at three level concentrations of low, medium and high range concentrations by addition of different amounts of standard mixture in methanol in different real water matrices, including industrial effluent, domestic waste, natural surface water and water from a gasoline station. Results can be seen in table 3.11-3.18.

The percent recovery was calculated via the external calibration curve and peak area. The external calibration curve was constructed as described in the section 2.2.4.2. Aqueous BTEX standard solution set B was used for the recovery test at low range BTEX concentrations and an aqueous BTEX standard solution set C was used for the recovery test at medium and high range BTEX concentrations.

2.7 Analysis of Real Samples

2.7.1 Water sample analysis

In this work, the optimized analytical method was applied for the determination of BTEX compounds in the real water samples, including drinking water, natural surface water collected near a vehicle traffic area in Chiang Mai. Both industrial effluent and industrial wastewater were collected from the Northern Industrial Estate of Thailand in Lumphun, domestic wastewater collected from Wastewater Treatment Facilities of Chiang Mai University and finally, a real water sample collected near a contaminated area of both gasoline station and motorcycle service garage were analyzed. No sample preparation was attempted. Only saturation of 10-ml of a real sample with 3 g NaCl was done before extraction and then the

extraction procedure and analysis using GC-FID under the optimized conditions were performed as in the Table 3.8. For the quantitative analysis of BTEX in the real water samples, it was carried out using the external calibration curve for each compound by GC-FID and confirmation of the compounds of interest were performed by GC-MS. Before construction of calibration curves, appropriate ranges were performed accordingly. Preparation of standard solutions used in constructing calibration curves for determination of BTEX in a real water sample from the motorcycle service garage and the industrial wastewater are described, as shown in Table 2.6 and Table 2.7, respectively. The samples were analyzed in triplicate. Concentrations of BTEX in the real water samples analyzed in this work are listed in Table 3.19. The resultant chromatograms by GC-FID and the identity of the peak identified by GC-MS are shown in Figure 3.40- 3.52.

2.7.2 Blood sample analysis

To apply this extraction method for such biological real samples, human blood sample was investigated with optimized HSSPME extraction and the identity of each of these BTEX was confirmed in the SPME extract by GC-MS but the other peaks were not identified. Figure 3.53 shows the total ion chromatogram and EI mass spectrum obtained from a blood sample by HSSPME with GC-MS final detection.