CHAPTER 4
DISCUSSION AND CONCLUSIONS

4.1 Discussion

The focus of this study is the development of a rapid and sensitive method for solid-phase microextraction of BTEX compounds, namely benzene, toluene, ethylbenzene, and xylene isomers, from real water samples. Determination was carried out using gas chromatography with flame ionization detection and confirmatory identification was attempted using a gas chromatograph coupled with a quadrupole mass spectrometer in the EI positive mode.

The chromatographic condition investigation was carried out in order to obtain adequate separation of six BTEXs with both reasonably shortest analysis time and good resolution possible. As a compomise for these, an HP-FFAP column (25 m x 0.32 mm I.D., 0.52 μm film thickness) with high polarity phase was chosen because it could separate nearly all the components. The optimal GC parameters appeared to be the column temperature held isothermally at 65 °C for 12 min, raised to 150 °C at 13 °C/min and held for 1.46 min, the carrier gas at flow rate of 1.0 ml/min, the injector maintained at 200 °C in the splitless mode. An SPME insert liner was used, and the FID detector was maintained at 220 °C.

For the GC conditions in the GC-MS system, a polar INNOWAX capillary column (30 m x 0.32 mm I.D., 0.32 μm film thickness) was used with the following optimal GC parameters: injection temperature 200 °C, splitless mode with SPME insert liner, the carrier gas at flow rate of 1.5 ml/min, the column temperature at 65 °C for 8 min, raised to 150 °C at 13 °C/min and held for 1.46 min.

The retention times of the BTEX standards are listed in Table 3.1 and typical GC-FID chromatograms resulting from the SPME analysis of the mixed BTEX standards in ultrapure water using 100 μm PDMS fiber are shown in Figures 3.1-3.3. Sampling from the liquid phase (direct SPME) and from the headspace above the liquid (headspace SPME) without any addition of salt before extraction yield results as shown in Figures 3.1 and 3.2, respectively. Both techniques gave identical results in terms of retention time and elution order of BTEX under optimum

conditions of SPME-GC-FID and HSSPME-GC-FID, respectively. The GC-FID chromatogram in Figure 3.3, obtained from HSSPME sampling with 3 g NaCl saturation before HSSPME extraction, shows identical elution order of BTEX, but different retention times of ethylbenzene and xylene isomers when compared to results from direct SPME and headspace SPME without any addition of salt before extraction as presented in Table 3.1. However, subsequent to identification and confirmation of BTEX peaks, the two methods were used in this work.

Firstly, in chromatographing the HSSPME extracts of each of individual BTEX standards and mixed BTEX standards in high purity methanol, the chromatographic patterns, as shown in Figure 3.4, indicated that the retention times of BTEX compounds were in good agreement in both cases.

In the second step, confirmatory identification of BTEX compounds was by means of HSSPME-GC-MS. HSSPME extracts from both saturated aqueous solution with salt and without salt addition, were analysed under optimum conditions of HS-SPME-GC-MS. Retention times of BTEX compounds with elution order are summarized in Table 3.2. GC-MS results obtained are illustrated in Figure 3.5-3.10. The total and ion chromatograms of BTEX in aqueous solution without salt addition are shown in Figure 3.5 whilst those of BTEX in aqueous solution with salt addition are shown in Figure 3.8. Figure 3.6 and 3.7 are mass spectra of individual BTEX for the former and Figure 3.9 and 3.10 are mass spectra of individual BTEX for the latter. Both sets gave identical results in terms of fragmentation pattern, i.e. the single ion of benzene at m/z = 78 ($[M]^+$ molecular ion) and characteristic fragments at m/z = 78 , 51 , 52 , 50 , 77 and 39 ; the single ion of toluene at m/z = 91 ($[M-H]^+$ ion) and characteristic fragments at m/z = 91 , 92 , 39 , 65 , 51 and 63; the single ion of ethylbenzene at m/z = 106 ($[M-Me]^+$ ion) and characteristic fragments at m/z = 91 , 106 , 51 , 39 , 65 and 77; the single ion of p-xylene at m/z = 106 ($[M-Me]^+$ ion) and characteristic fragments at m/z = 91 , 106 , 105 , 77 , 51 and 39; the single ion of m-xylene at m/z = 106 ($[M-Me]^+$ ion) and characteristic fragments at m/z = 91 , 106 , 105 , 39 , 51 and 77; and the single ion of o-xylene at m/z = 106 ($[M-Me]^+$ ion) and characteristic fragments at m/z = 91 , 106 , 39 , 105 , 51 and 71.

In this work, it was found that the elution order (retention time) of BTEX compounds was benzene < toluene < ethylbenzene < p-xylene < m-xylene < o-xylene. This elution order could be explained in terms of boiling point and molecular weight of BTEX compounds. High boiling point and high molecular weight caused the BTEX to be retained on the column longer.

In the initial development of the SPME method for the determination of BTEX compounds in water, it was carried out with a commercially available polydimethylsiloxane fiber having a film thickness of 100 μm, housed in its manual holder. There are several important reasons why this material was chosen. Because the affinity of the fiber for an analyte is the most important factor in SPME, the principle of "like dissolves like" applies [64]. Polydimethylsiloxane is a non polar stationary phase which retains hydrocarbons very well [28]. It is an excellent coating for sampling a broad range of analytes [65] and it is also thermally stable GC stationary phase, up to about 300 °C [37]. Although PDMS fibers with thinner films equilibrate faster, they were not investigated in this work as they have a lower capacity which is not useful for analyzing highly volatile trace level compounds [35]. In addition, sensitivity is improved by increasing the thickness of the coating as the volume of coating increases [28,32].

The typical fiber was conditioned at 250 °C for 1 h in the GC injector port before use. Desorption temperature at 200 °C was used for 2 min for all the BTEX components in real water samples, and there was no sign of carryover between samples.

Two sample techniques were investigated. One involved immersing the 100 μm PDMS coated fiber in the aqueous phase (SPME) and, in the other, the fiber was suspended in the headspace above the water (HSSPME). Results obtained are shown in Table 3.3 and summarized in the bar graph form in Figure 3.11. Both techniques gave identical results for most of the compounds. Sampling the headspace presents also a significant advantage in terms of selectivity because only the volatiles and semivolatiles are released into the headspace. Since the fiber is not in contact with the real sample, background adsorption and matrix effect are reduced, resulting in the enhancement of the life expectancy of SPME fiber. All subsequent experiments were

therefore performed with the fiber suspended in the headspace above the water (HSSPME).

The effect of stirring speed with conventional magnetic stirring was optimized and adjusted to the maximum rate to accelerate adsorption [66]. When water is stirred rapidly to enhance the mass transfer of analytes, the analytes must diffuse through the water before they can be adsorbed by the fiber coating [28]. Table 3.4 shows the GC areas obtained at different agitation speeds expressed as % power of maximum speed and the curves obtained in Figure 3.12 show clearly that with no agitation a very poor extraction is achieved and this extraction increases with increased stirring rate. However, above 80% of the maximum speed the stir bar begins to vibrate and agitation of the solution is worse. Frequency, the base plate may heat up during stirrer operation, resulting in change of the distribution constant, which affects reproducibility of the measurement precision. Thus, the rest of the experiments were carried out at this optimum stirring rate (70% of maximum speed).

The effect of ionic strength on the headspace BTEX determination was studied by saturating the aqueous solution before extraction with 3 g NaCl and checked by comparison of signals when the sample was saturated with 3 g NaCl and that obtained without adding salt. Results obtained demonstrated that saturation of the aqueous phase with 3 g NaCl increased the extraction efficiency for most of the BTEX compounds. The results are presented in Table 3.5 and as a bar graph illustrating in Figure 3.13. Because the partition coefficients of analytes are partially determined by the reaction between target analytes and the matrix, the nature of the matrix can be modified to influence the coating/matrix partition coefficients of the analytes. By adding sodium chloride or any suitable salt, e.g. potassium chloride, to aqueous samples, the ionic strength of water can be increased, thereby increasing the partitioning of polar organic compounds (but not ions) into the polymer coating. As the neutral forms of analytes are more efficiently extracted by the non-ionic polymeric coating [28,32], salt was added to the aqueous solution for all subsequent experiments. For this study "salting-out effect" decreases the solubility of analytes and thus increases the adsorption.

The adsorption-time profile was studied by monitoring the GC-FID area counts as a function of exposure time of the fiber to the sample from 3 to 20 min.

Table 3.6 represents the performance of a 100 μm PDMS fiber for the six BTEXs . The increased equilibrium period usually increasing distribution constant of the analyte [51]. Figure 3.14 shows the adsorption time profiles for the six BTEXs in an aqueous solution agitated by magnetic bar stirring. None of the six chemicals reached an adsorption equilibrium within 20 min of sampling. Although at equilibrium, the SPME sampling method has the maximum sensitivity, for a practical purpose, the two factors other than the time required for equilibrium are precision and sensitivity [68]. Theoretical treatment presented in equations 1.3 and 1.7 in Section 1.2.3 suggest that a linear proportional relationship exits between the adsorbed analyte (C_f) and its initial concentration in the sample matrix (C_o). If the adsorption time and agitation conditions are held constant throughout the experiment, it is not necessary to reach for quantitative analysis [52,66-67].

In addition, the BTEX adsorption-time profile on three of 100 μm PDMS fibers were also investigated by varying the exposure time between 3 and 12 min into three level concentrations of an aqueous mixed BTEX standard solution. The results presented in Figures 3.15-3.20 indicated that the use of the equilibrium time in the adsorption phase can be 10 min because sensitivities obtained are acceptable and linear proportional relationship between the adsorbed analyte and its initial concentration in the sample matrix of six chemicals of BTEXs exists. All subsequent experiments were therefore performed with adsorption time about 10 min optimized for HSSPME.

The effect of extraction temperature in the adsorption of BTEX into a 100 μm PDMS fiber by HSSPME was investigated in a temperature range 2-40 °C. The results are presented in Figure 3.21.An increase in temperature during the extraction process enhances the diffusion of analytes towards the fiber, decreasing the time needed to reach the equilibrium [8,32]. However, in this work the temperature was found to have a significant negative effect on the BTEX compounds adsorption, so, it seems to be better to work in a lower temperature range. This can be explained by the exothermic adsorption process by which the BTEX compounds are partitioned between the headspace and the PDMS coating. A higher temperature increases the concentration of the BTEXs in the headspace by decreasing the partition coefficient

between the PDMS coating and the headspace [21]. As a result of this, the procedure of the experiments would be easier to perform at the temperature of the laboratory (ambient temperature: 24-25°C). It has been reported that better conditions can be obtained by heating the sample and internally cooling the fiber [22,24] to improve the analyte diffusion and to favour the exothermic process ; however, such a system is difficult to realize and real benefits are poor.

The effect of temperature as illustrated in Figure 3.21 cab be best explained via the following equation [32] :

$$K_{fs} = K_o \exp [-\Delta H/R] [(1/T) - (1/T_o)] \qquad (4.1)$$

where K_{fs} is the partition coefficient between the sample and fiber coating , K_o is the distribution constant when both fiber and sample are at temperature To (in Kelvin), ΔH is the molar change in enthalpy of analyte when it moves from sample to fiber coating, and R is the gas constant, It is obvious from this equation that raising the temperature will decrease K_{fs}.

After establishing the optimized conditions of HSSPME-GC-FID for the determination of BTEX compounds in real water samples, the results are summarized in Table 3.8. The detection limits, linearity and precision were investigated by extracting artificially spiked ultrapure water with standard BTEXs and the results are summarized in Table 3.10.

The detection limit, defined as the peak height of concentration of an analyte in an aqueous solution giving rise to a peak height with a signal-to-noise ratio of three. Table 3.9 compares the limit of detection of BTEX in water. As this table indicates, the obtained LOD for toluene, ethylbenzene and xylene isomers could have the lower detection limit because they have the higher affinity for the fiber coating, as indicated by its distribution constant (K) and the octanol-water partition coefficients (K_{ow}). However , the experimental and literature values [2,19] studied indicate comparable relationship between experimental distribution constants (K) and literature values for the octanol-water partition coefficients (K_{ow}) and also the same order of magnitude and trend in the same direction. Table 4.1 shows the relationship of experimental distribution constants (K) and literature values for the octanol-water

partition coefficients (K_{ow}) of the BTEX components in water [19]. Arthur *et al.* [20] reported that the differences in minimum detection limits for the BTEX components reflect the trend in the octanol-water partition coefficients (K_{ow}).

Table 4.1 Experimental distribution constants (K) and literature values for the octanol-water partition coefficients (K_{ow}) of BTEX compounds in water [19].

BTEX component	$Log_{10} K$	$Log_{10} K_{ow}$
Benzene	2.30	2.13
Toluene	2.88	2.69
Ethylbenzene	3.33	2.84
p-Xylene	3.31	3.15
m-Xylene	3.31	3.20
o-Xylene	3.26	2.77

To evaluate the linearity of this work, Figures 3.22-3.27 present the slopes, correlation coefficients and excellent linear ranges in the concentration range from 1 – 1,000 μg/L for BTEX, except for benzene (2 – 2,000 μg/L). All the compounds studied were characterised by regression coefficients better than 0.999.

The precision of optimized method was evaluated at low level concentrations of BTEX. The R.S.D. values were found to be between 4 to 9%, as presented in Table 3.10.

Recovery assay was investigated under the optimal conditions of HS-SPME-GC-FID of this research work. Efficiency of the method was investigated by spiking BTEXs at three level concentrations into the real samples from different sources, including, gasoline station, industrial effluent, natural surface water and domestic wastewater. With spiking at low level concentrations, the estimated concentrations and percent recovery data with the R.S.D. (n=3) values are shown in Tables 3.11-3.14. Chromatograms of water from gasoline station, industrial effluent,

domestic wastewater, and natural surface water are shown in Figures 3.28 , 3.30 , 3.32 and 3.34, respectively.

Spiking at medium and high level concentrations into real water samples was applied to the determination of BTEXs in industrial effluent and domestic wastewater. The estimated concentrations and percent recovery data with the R.S.D. (n=3) values are shown in Table 3.15-3.18. Chromatograms of industrial effluent and domestic wastewater at medium , and high level concentrations are shown in Figures 3.36 and 3.37 and Figures 3.38 and 3.39, respectively.

For the spiking at low, medium and high level concentrations, the percent recoveries of most BTEX were found to be higher than 61% with RSD in the range 0.4-13.1%, 70% with RSD in the range 1.3-10.2% and 73% with RSD in the range 1.2-4.6% , respectively.

It should be noted that the LOD determination in this work simply based on the criterion that a signal-to-noise ratio is of a minimum 3:1 and the procedure by Miller and Miller [69] was not adopted due to practical reasons.

In order to assess the practical utility of the HSSPME-GC-FID method, the method was applied to the determination of BTEX compounds in real water samples. The results are summarized in Table 3.19. The chromatograms obtained from blank ultrapure water, drinking water, natural surface water, industrial effluent industrial wastewater, domestic wastewater, and water sample collected from a gasoline station and a motorcycle service garage are shown in Figures 3.40 , 3.41 , 3.42 , 3.43 , 3.44 , 3.47 3.48 and 3.49, respectively.

The confirmation of BTEX compounds in real water samples collected from industrial wastewater and a motorbike service garage was attemped using the HS-SPME-GC-MS method. Figure 3.46 shows the GC-MS total ion chromatogram and also the EI mass spectrum patterns of the peak toluene in real water samples collected from industrial wastewater, indicating the characteristic fragments of toluene at m/z = 91 , 92 , 39 , 65 , 51 and 63. Figures 3.51 and 3.52 show the total ion chromatogram and the EI mass spectrum patterns of the peaks toluene, ethylbenzene, xylene isomers in a real water sample collected from the motorcycle service garage indicating the characteristic fragments of toluene at m/z = 91 , 92 , 39 , 65 , 51 and 63 , of ethylbenzene at m/z = 91 , 106 , 51 , 39 , 65 and 77 , of p-xylene at m/z = 91 , 106 ,

105 , 77 , 51 and 39 , of m-xylene at m/z = 91 , 106 , 105 , 39 , 51 and 77 and of o-xylene at m/z = 91 , 106 , 39 , 105 51 and 71.

To further demonstrate the advances of the developed HSSPME-GC-MS method for the screening and quantification of BTEX compounds in a biological specimen, such as human blood provided by Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, the sample was tested using the proposed HS-SPME-GC-MS method. Figure 3.53 shows the total ion chromatogram of the human blood sample and EI mass spectrum pattern of toluene at m/z = 91 , 92 , 39 , 65 , 51 and 63. This trial examination indicates that the developed HSSPME-GC-MS would be suitable for the BTEX determination in biological samples.

4.2 **Conclusions**

The optimized headspace solid-phase microextraction method described in this work was found to be applicable to the determination of all six components of BTEX compounds consisting of benzene, toluene, ethylbenzene, p-xylene, m-xylene and o-xylene in real water samples with both polar phases of HP-FFAP and INNOWAX capillary column by GC-FID and GC-MS.

The optimal conditions of the solid-phase microextraction (SPME) using 100 μm poly(dimethylsiloxane) fiber coating employed include the following: Headspace SPME sampling mode (HSSPME) above an 10 ml-aliquot of aqueous solution saturated with 3 g NaCl in a 22-ml vial, agitated with magnetic stirring bar under constant stirring rate at 70% of maximum speed, 100 μm PDMS fiber coating with 10 min adsorbing time at ambient temperature extraction (24-25 °C), thermal desorption time of 2 min into injection port of GC at 200 °C. Subsequently, the quantitation was performed on GC-FID with an HP-FFAP capillary column, the temperature program was 65 °C (12 min) -150 °C (13 °C/min)–150 °C (1.46 min) at carrier gas flow rate 1.0 ml/min. This HSSPME-GC-FID optimal method could be used to detect all six components of BTEXs in artificially spiked ultrapure water samples at 0.5-1.0 ng/ml levels in 10 ml water samples. External calibration curves were found to be linear in the range 1 – 1,000 ng/ml of BTEX except for benzene

(2 – 2,000 ng/ml), with a correlation coefficient better than 0.999 and coefficient of variation of 1-9%.

After employing the optimal conditions in the analysis of the environmental real water samples, the HS-SPME-GC-MS was employed as the definitive procedure for confirmation. Toluene was detected in industrial wastewater collected from the Northern Industrial Estate in Lamphun Province at the concentration of 116.8 μg/L (6.2% R.S.D.). Toluene, ethylbenzene, p-xylene, m-xylene and o-xylene were detected in the real water samples collected from a contaminated area near the motorcycle service garage in Chiang Mai Province at the concentration of 7.2 μg/L (1.1% R.S.D.), 1.4 μg/L (3.3% R.S.D.), 2.0 μg/L (1.1% R.S.D.), 2.3 μg/L (2.2% R.S.D.) and 1.7 μg/L (2.9% R.S.D.), respectively. No BTEXs were detected in real water samples from drinking water, natural surface water, industrial effluent, domestic wastewater and water collected from location near a gasoline station.

As for the use of this SPME developed method, further studies should be investigated for toxicological and forensic use to minimize the cost and rapid screening for BTEX determination in the biological monitoring of people environmentally exposed to BTEX in urban areas, since BTEX are likely to become a serious environmental problem as these compounds are hazardous, carcinogenic and neurotoxic compounds.