CHAPTER IV

RESULTS AND DISCUSSION

4.1 Gas chromatographic conditions

The standard solution of 200 μg/L THMs in 1-octanol was injected according to the GC condition in Table 3.4. The chromatogram and resolution of standard THMs were shown in Figure 4.1 and Table 4.1, respectively.
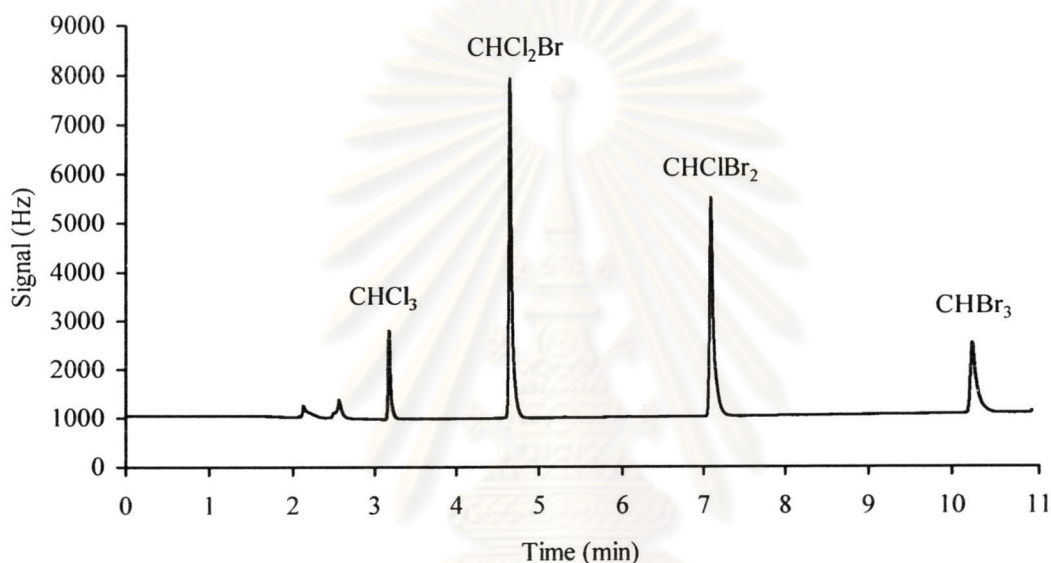

Figure 4.1 The chromatogram of standard THMs under GC condition in Table 3.4.

Table 4.1 Retention time and resolution of standard THMs under GC condition in Table 3.4.

Analyte	Retention time (min)	Resolution
Chloroform ($CHCl_3$)	3.24	
Bromodichloromethane ($CHCl_2Br$)	4.72	22.16
Chlorodibromomethane ($CHClBr_2$)	7.18	29.33
Bromoform ($CHBr_3$)	10.34	28.14

The standard solution of 200-2000 µg/L haloacetic methyl ester in dodecane was injected according to the GC condition in Table 3.5. The chromatogram and resolution of standard HAAs were shown in Figure 4.2 and Table 4.2, respectively.
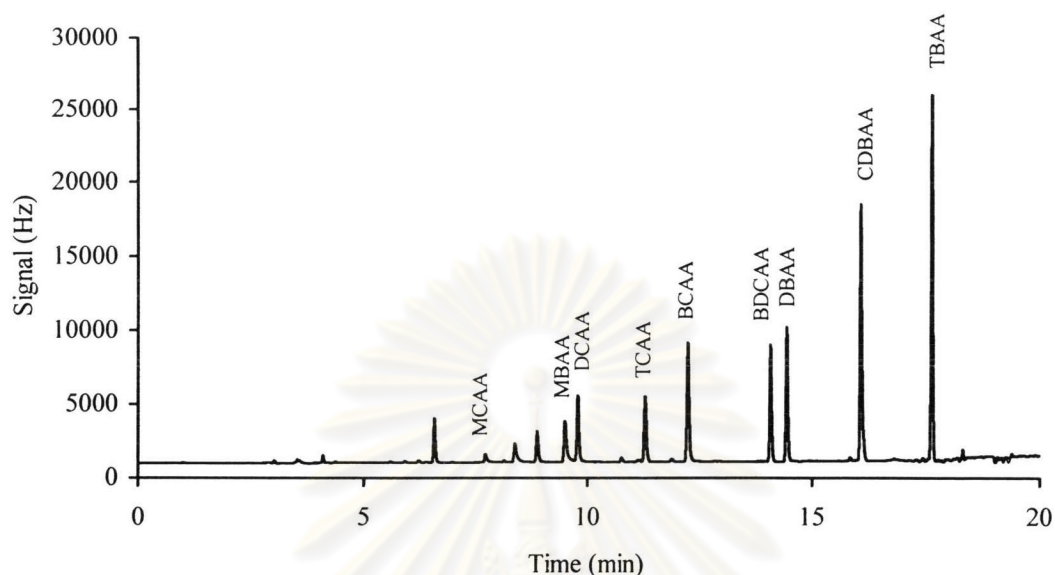

Figure 4.2 The chromatograms of standard haloacetic methyl ester under GC condition in Table 3.5.

Table 4.2 Retention time and resolution of standard haloacetic methyl ester under GC condition in Table 3.5.

Analyte	Retention time (min)	Resolution
Monochloroacetic acid (MCAA)	7.66	
Monobromoacetic acid (MBAA)	9.47	12.89
Dichloroacetic acid (DCAA)	9.76	2.16
Trichloroacetic acid (TCAA)	11.30	13.64
Bromochloroacetic acid (BCAA)	12.24	8.22
Bromodichloroacetic acid (BDCAA)	14.07	17.09
Dibromoacetic acid (DBAA)	14.42	3.73
Chlorodibromoacetic acid (CDBAA)	16.03	16.24
Tribromoacetic acid (TBAA)	17.59	15.34

The GC condition in Table 3.4 and Table 3.5 can be used to separate all of the THMs and HAAs, respectively, because the resolution of each peak in Table 4.1 and Table 4.2 are graters than 1.5.

4.2 Determination of immobilized organic solvent volume

The results of the immobilized organic solvent volume in the pores of hollow fiber membrane were shown in Table 4.3.

Table 4.3 Volume of 1-octanol in the pore of polypropylene and polysulfone hollow fiber membrane.

	Volume of 1-octanol in the pores (µL)	
	Polypropylene* (n = 37)	Polysulfone* (n = 31)
Mean	27.8	23.9
Standard deviation	0.5	3.1
Relative standard deviation (%)	2	13

* The length of PP and PS were 8.5 cm and 7 cm, respectively.

The results indicate that the volume of 1-octanol in the pores of PP and PS hollow fiber membrane were consistent, with the RSD values below 13 %. This shows that the preparation of hollow fiber membrane show high repeatability. The RSD of PS was relatively higher than that of PP. This may be due to that fiber to fiber variations of PS is higher than PP. In order to compare extraction efficiency of hollow fiber membrane, total volume of organic solvent of PP should be similarity to PS, the volume of organic solvent in the pores was calculated to set length of hollow fiber membrane in Section 4.3.1.

4.3 Liquid-phase microextraction optimization for THMs extraction

4.3.1 Type of hollow fiber membrane

The hollow fiber membrane employed in LPME should be hydrophobic as well as compatible with the organic solvent being used. The most common type of hollow fiber membrane used in LPME is polypropylene capillary membranes because it is compatible with a broad range of organic solvent [50,52]. In this study, another type of hollow fiber membrane, i.e., polysulfone hollow fiber membrane, which was locally available for water purification system was explored, in order to compare the extraction performance with the commonly used polypropylene membrane. The extraction performance was shown in Figure 4.3.
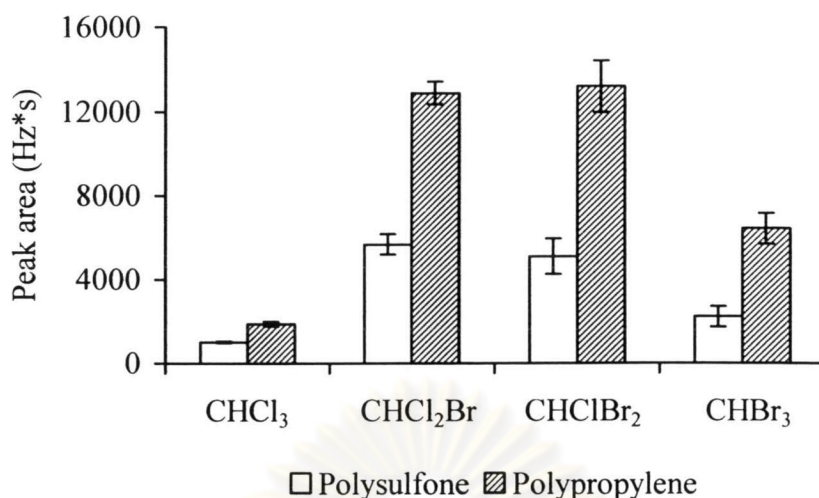
Figure 4.3 Comparison of liquid-phase microextraction extraction using polysulfone and polypropylene hollow fiber membrane (THMs: 10 µg/L, direct extraction, extracting solvent: 1-octanol, extraction time: 30 min, extraction temperature: 35 °C, n = 3).

It was found that the polysulfone membrane showed poorer extraction efficiency than polypropylene membrane. Since the rate of extraction was limited by the diffusion of the analyte through the acceptor solution in the walls of the fiber, as of the same extraction time, the smaller porosity (PP = 66 %, PS = 30 %) and the greater wall thickness of the polysulfone membrane (PP = 200 µm, PS = 270 µm) provided less contact area and virtually created the thick diffusion layer through the wall of the fiber resulting in smaller extraction [91,92].

4.3.2 Effect of organic solvent

Selection of organic solvent for liquid-phase microextraction is important. The solvent should not only be able to extract analyte, but also should be compatible with the fiber, low solubility in water to prevent dissolution into the aqueous phase, low volatility to prevent evaporation during extraction and separated from the analyte peak in chromatogram. In this study, three solvents such as dodecane, 1-octanol and dihexyl ether were examined. The results presented in Figure 4.4 indicated that dodecane exhibited the worst extraction efficiency while the extraction efficiencies using dihexyl ether and 1-octanol were better and satisfactory. However, the chromatographic peaks of THMs in dihexyl ether were poor showing shoulders (Figure 4.5). So 1-octanol was selected as the extraction solvent for all subsequent experiments.
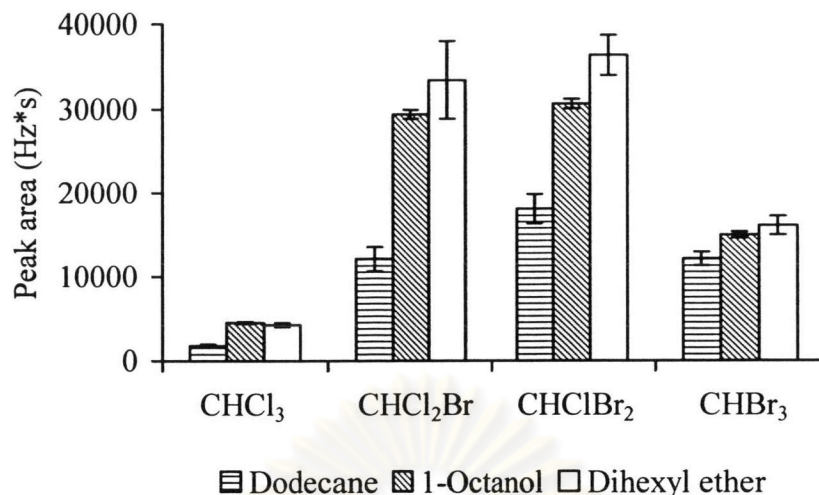
Figure 4.4 Comparison of liquid-phase microextraction efficiencies of THMs using different organic solvents (THMs: 10 µg/L, direct extraction, extraction time: 30 min, extraction temperature: 35 °C, n = 3).
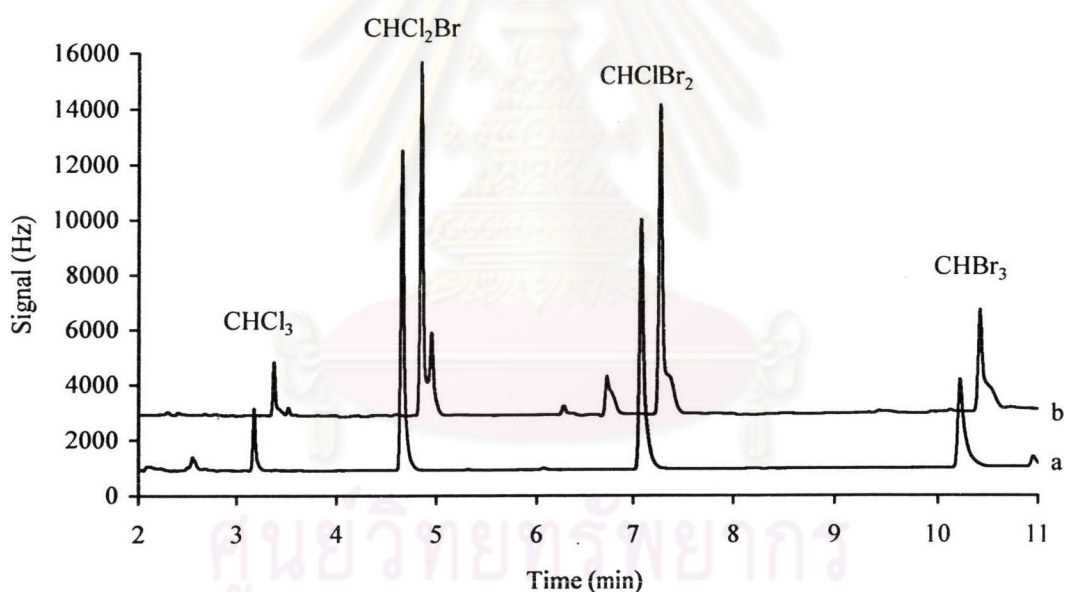

Figure 4.5 Chromatogram of liquid-phase microextraction of THMs using (a) 1-octanol; (b) dihexyl ether.

4.3.3 Extraction mode

Since THMs are volatile as well as soluble in water, two modes of LPME extraction can be performed; direct extraction and headspace extraction. This study, two modes of LPME were employed and compared extraction efficiencies. The results obtained for each THMs with different extraction modes were shown in Figure 4.6.
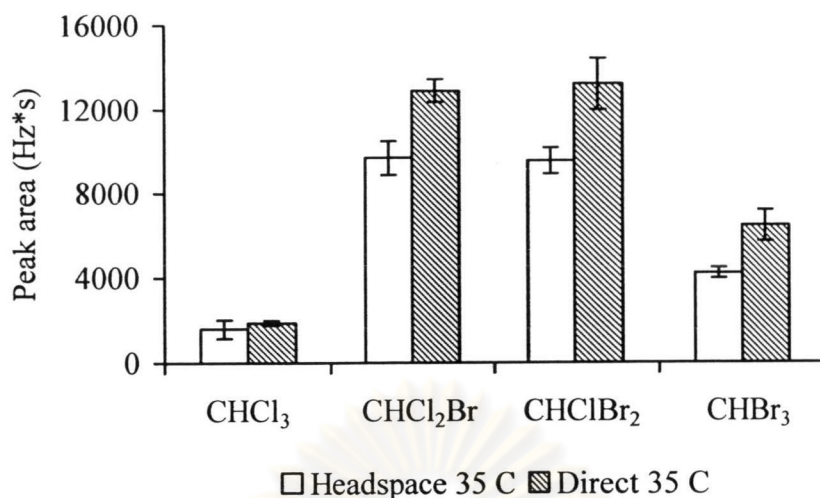
Figure 4.6 Extraction efficiencies of THMs using direct and headspace sampling mode (THMs: 10 µg/L, extraction time: 30 min, extraction temperature: 35 °C, n = 3).

In direct extraction mode, LPME was directly immersed into the sample and the analytes was diffused directly from the water sample into the extracting solvent. In the headspace mode, LPME was hanged in the headspace. The analytes need to be transported through the barrier of air before diffused into the extracting phase. Although the mass transfer of the analytes in the headspace is typically a fast process because the diffusion coefficient in the gas phase is about 10^4 times greater than the diffusion coefficient in liquid media [93], the result in **Figure 4.6** showed that the amounts of analytes absorbed in the headspace extraction mode were less than direct extraction mode. It is probably due to that the mass extracted depends upon the temperature that limited mass transfer of the analytes in the aqueous phase into the headspace. To test this hypothesis, extraction temperature in headspace mode was increased from 35 °C to 65 °C. It can be seen from Figure 4.7 that the sensitivity of the method increased with elevating temperature. This can be explained by the facts that at high temperature, the vapor pressure of the analytes in the headspace are increased; consequently the amounts of analytes in the headspace were increased. Despite the fact that at 65 °C the extraction efficiency was the highest, many water droplets were formed on the outer surface of the hollow fiber, which could affect the extraction reproducibility. At 50 °C the sensitivity was slightly larger than direct extraction at 35 °C. Since the experiment was intended to be simple, direct extraction at 35 °C was chosen in this study.
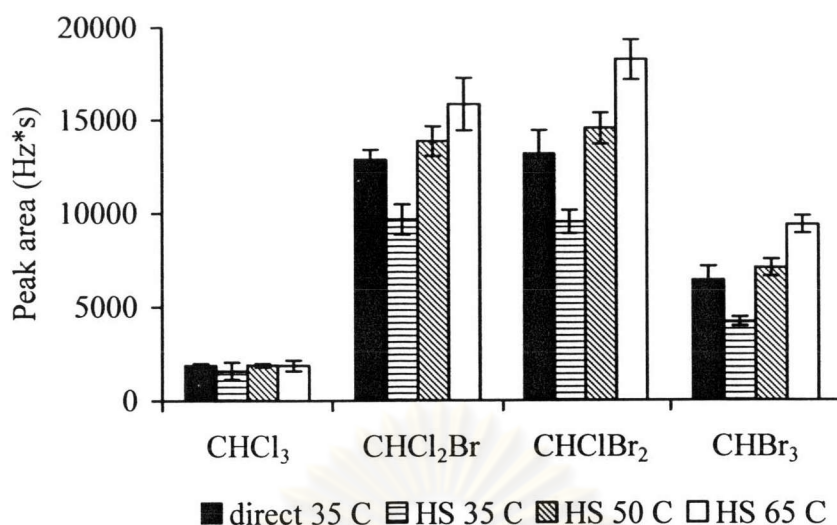
Figure 4.7 Effect of temperature on headspace extraction (THMs: 10 μg/L, extraction time: 30 min, n = 3).

4.3.4 Extraction time

Owing to that liquid-phase microextraction is not an exhaustive extraction method, the amounts of extracted analytes are expected to increase with exposure times. As illustrated in Figure 4.8, the extraction gradually increased with increasing exposure times.
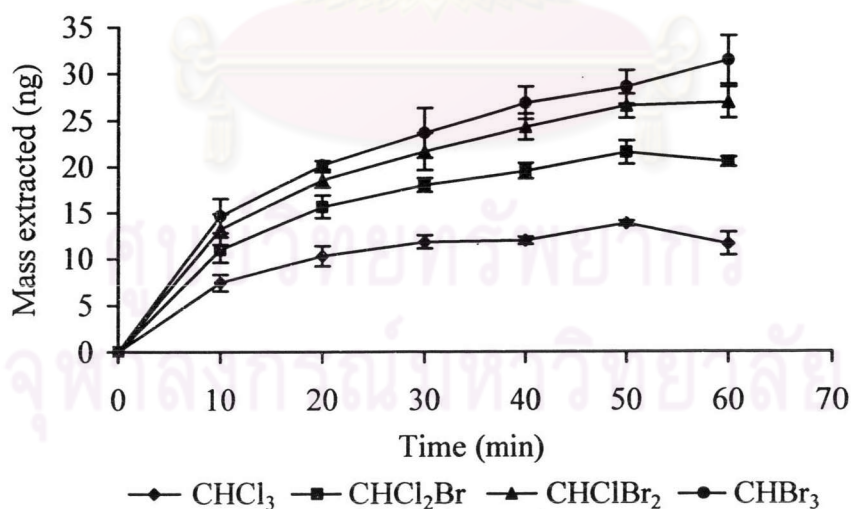

Figure 4.8 Extraction time profile for liquid-phase microextraction of THMs in water samples (THMs: 10 μg/L, direct extraction, extraction temperature: 35 °C, n = 3).

It seemed that chloroform had reached equilibrium after 20 min, dichlorobromomethane and dibromochloromethane had reached equilibrium after 50 min, and bromoform had not yet reached equilibrium after 60 min. Since it was non stirring condition the depleting zone theory similar to non agitation SPME may be applied. As the distance from the membrane surface increased the depleting zone surrounding the membrane might have formed. Transport of analyte from the progressively thicker depleted layer to the extracting solvent determines overall extraction speed. The more analytes need to be transported to the extracting solvent, the longer the process will last [90]. For routine analysis, it is not necessary to attain equilibrium if constant extracting conditions are maintained and the desired sensitivity is met [94]. Since the estimated sensitivity of all analytes has satisfactorily achieved at 30 min, the extraction time of 30 min was chosen for all subsequent experiments.

4.3.5 Influence of sample volume on enrichment factor

As shown in equation 2.15, the volume ratio of extracting solvent to sample is an important factor on enrichment factor. Two sample sizes of 10 ml and 20 were experimented. As illustrated in Figure 4.9, the enrichment factor was not much increased when the sample volumes were increased by the factor of 2. As sample volume increased, more amount of analyte was available for extraction. However, since the formation of the depleting zone, only a few more amount of analyte had been extracted. For this reason, the concentration of analyte extracted was slightly increased while the bulk concentration was virtually unchanged resulting in slightly increased enrichment factor. A sample volume of 10 mL was adopted in the following studies since lower sample volume resulted in convenient operation for extraction.
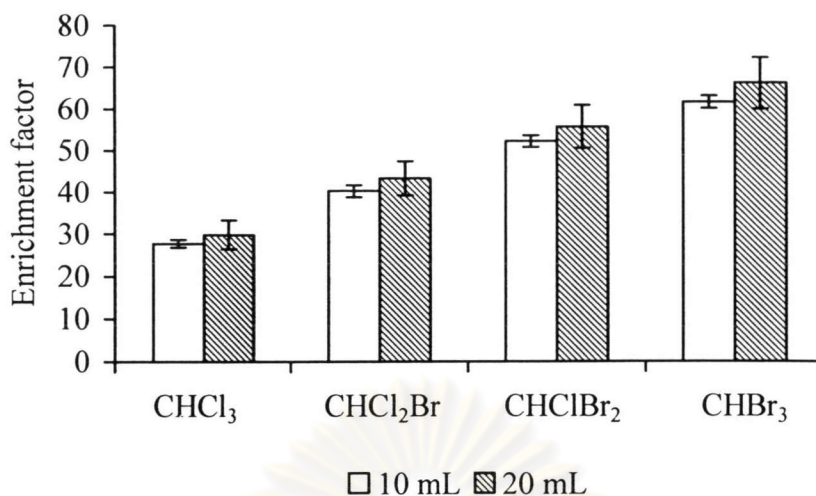
Figure 4.9 Influence of sample volumes on the enrichment factors of THMs (THMs: 5 μg/L, direct extraction, extraction time: 30 min, extraction temperature: 35 °C, n = 5).

Table 4.4 showed extraction efficiencies and enrichment factors of THMs obtained from LPME and LLE. Extraction efficiencies and enrichment factors of LLE were calculated by Equation 2.14 and 2.15. Because of the large volume of the solvent used, LLE provides high recoveries. However, analyte enrichments were relatively low. In comparison, LLE provides high recovery, whereas LPME provides high enrichment.

Table 4.4 Calculated extraction efficiencies and enrichment factors of THMs in LPME and LLE.

Analyte	Extraction efficiency (%)		Enrichment factor	
	LPME[a]	LLE[b]	LPME[a]	LLE[b]
CHCl$_3$	15	90	28	9
CHCl$_2$Br	21	92	40	9
CHClBr$_2$	28	95	52	9
CHBr$_3$	32	96	62	10

[a] Calculated from experiment (sample: 10 mL, direct extraction, 1-octanol volume: 0.053 mL, extraction time: 30 min, temperature: 35 °C).

[b] Based on US EPA method 551.1 (sample: 50 mL, 1-octanol volume: 5 mL). Log *P* was shown in Table 2.3.

4.4 Method evaluation of trihalomethanes

Calibration curves for all THMs were established in the concentration range from 0.2 to 100 μg/L. As shown in Table 4.5, the linearity for liquid-phase microextraction was very good with correlation coefficients (R^2) being greater than 0.9968 (n = 7). The method detection limit (MDL) and the method quantification limit (MQL) based on S/N = 3 and 10 were between 0.01 to 0.2 μg/L and 0.04 to 0.7 μg/L, respectively. The MDL of all THMs was found in low μg/L level and much lower than the MCL values of WHO, EU and US EPA regulations. In comparison with previously reported headspace SPME method [28] and headspace SDME [47], lower MDL for all four targeted analytes was obtained.

Table 4.5 Linear ranges, correlation coefficients (R^2) and method detection limit of liquid-phase microextraction of THMs in water samples determined by GC-μECD.

Analyte	Linear range (μg/L)	R^2	MDL (μg/L)	MQL (μg/L)	HS-SPME [28] MDL (μg/L)	HS-SDME [47] MDL (μg/L)
$CHCl_3$	1.0-100	0.9973	0.2	0.7	2.8	0.40
$CHCl_2Br$	0.2-100	0.9984	0.01	0.04	1.4	0.15
$CHClBr_2$	0.2-100	0.9982	0.01	0.05	1.0	0.15
$CHBr_3$	0.2-100	0.9968	0.04	0.1	1.2	0.20

The accuracy and precision of the method were expressed in recovery and % relative standard deviation (%RSD) of analyzing replicated spiked water samples at various concentrations as shown in Table 4.6. The recoveries from spiked concentration range from 5 to 50 μg/L were 96 to 113 % and from 1 μg/L were 62 to 130 % with % RSD less than 7 % (the acceptable values of RSD at concentration of 50 μg/L are not greater than 25 %) [95].

Table 4.6 % Recoveries and % relative standard deviations of THMs spiked water samples at concentration range from 1 to 50 µg/L.

Analyte	Recovery (%RSD)			
	1 µg/L (n = 5)	5 µg/L (n = 5)	10 µg/L (n = 3)	50 µg/L (n = 5)
$CHCl_3$	62 (2)	98 (3)	113 (3)	98 (7)
$CHCl_2Br$	130 (5)	105 (4)	107 (2)	98 (5)
$CHClBr_2$	105 (5)	102 (3)	103 (2)	96 (6)
$CHBr_3$	117 (6)	100 (2)	105 (2)	97 (6)

4.5 Liquid-phase microextraction optimization for HAAs extraction

4.5.1 Effect of organic solvent

In this study, three solvents i.e. dodecane, 1-octanol and dihexyl ether were used to compare the extraction efficiency. The result was shown in Figure 4.10.

Figure 4.10 Extraction efficiency of various organic solvent (MeOH: 1 mL, H_2SO_4: 1 mL, extraction time: 60 min, extraction temperature: 50 ºC, n = 3).

The experiments indicated that extraction efficiency were significantly different for each solvent. All solvents in this experiment were not be able to extract all nine HAAs. Five HAAs (i.e. MCAA, MBAA, DCAA, TCAA and DBAA) that are regulated under US EPA guidelines were able to be extracted by dihexyl ether and 1-octanol. However, when consider six HAAs (i.e. HAA5 + BCAA) that are regulated under the US

EPA Information Collection Rule (ICR), dihexyl ether could not show BCAA. This is because interference peak from an impurity in the dihexyl ether. Therefore, 1-octanol was chosen as the extraction solvent in this study. The chromatogram of blank and HAAs in octanol after extracted by LPME were presented in Figure 4.11.
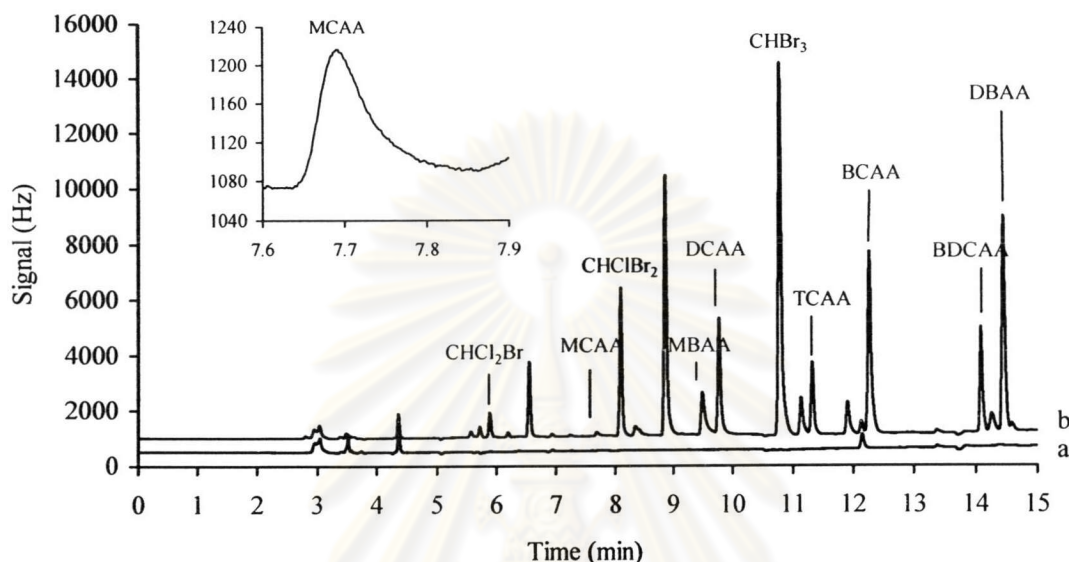

Figure 4.11 Chromatograms of headspace liquid-phase microextraction (a) blank; (b) spiked water sample containing 90 µg/L MCAA, 60 µg/L MBAA, 90 µg/L DCAA, 30 µg/L TCAA, 60 µg/L BCAA, 60 µg/L BDCAA and 30 µg/L DBAA.

It can be observed that there were peaks of THMs appeared in the chromatogram. It is reasonable to assume that decarboxylation of HAAs during extraction results in the formation of corresponding THMs. For example, TBAA could form tribromomethane as reaction below [79]:

$$Br_3CCOOH \longrightarrow CHBr_3 + CO_2$$

4.5.2 Effect of extraction temperature

The effect of extraction temperature was examined from 40-65 $^{\circ}$C. The results were shown in Figure 4.12.
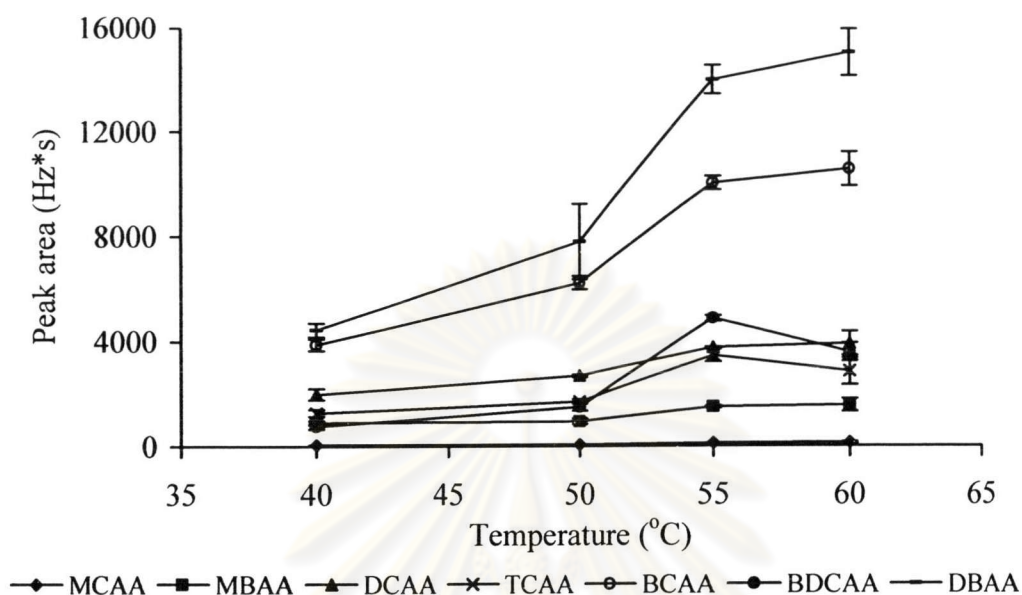

Figure 4.12 Effect of extraction temperature on the extraction efficiency (MeOH: 1 mL, H_2SO_4: 1 mL, extraction time: 60 min, n = 3).

It can be observed that the amount of analytes extracted into the extracting solvent increases with increasing temperature up to 60 $^{\circ}$C. This can be explained by the fact that higher temperature may increase esterification efficiency [18] and also increase the amounts of analytes in the headspace resulting in increased extracted amounts of analytes. Above 60 $^{\circ}$C, the extraction could not be achieved. This was possibly caused by high vapor pressure of methanol in the headspace, which could dissolve into 1-octanol in the membrane. Consequently, 1-octanol in the lumen was push by the methanol out of the hollow fiber at the top of needle. In this study, 55 $^{\circ}$C was selected for optimum temperature owing to after 55 $^{\circ}$C the curves became flat and the peak areas slightly increased.

4.5.3 Effect of methanol volume on in-situ derivatization

As it can be seen from Figure 4.13, the peak areas of HAAs increased with increasing methanol volumes up to 1 mL. This can be explained by the fact that methanol may shift the equilibrium of the esterification reaction towards ester in in-situ derivatization of HAA in water. However, the peak areas of analytes decreased by further

increasing methanol volumes from 1 to 1.25 mL. This may be due to that ester derivatives can be dissolved in methanol; consequently, volatilization of the ester derivatives from aqueous solution to the headspace was decreased. Hence, the optimum volume of methanol was 1 mL.
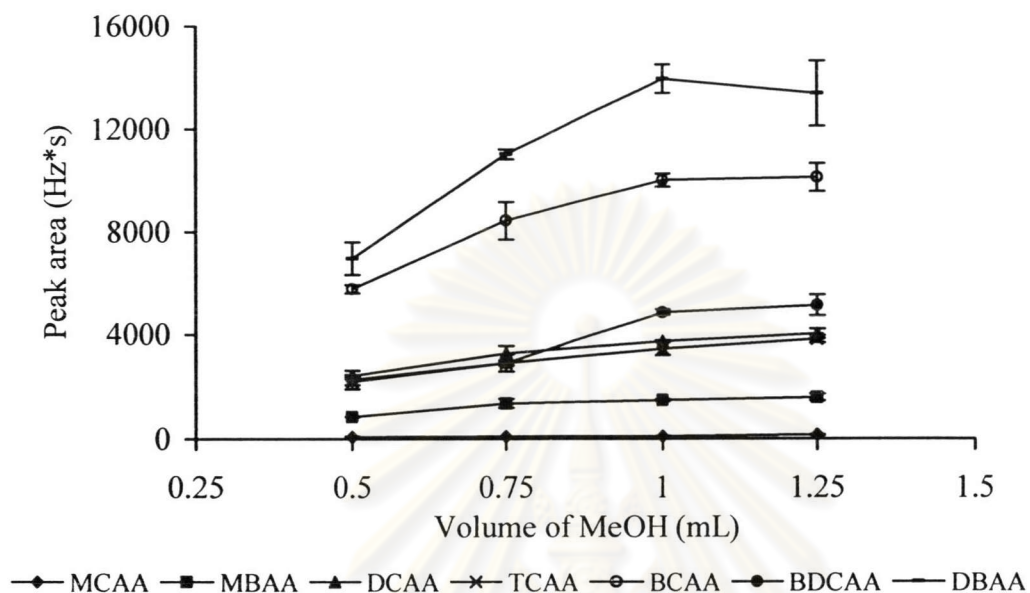

Figure 4.13 Effect of volume of methanol on extraction efficiency (H_2SO_4: 1 mL, extraction time: 60 min, extraction temperature: 55 °C, n = 3).

4.5.4 Extraction time

The extraction time profiles were investigated by monitoring the mass extracted with exposure time. The mass extracted obtained for each HAAs with different extraction times were shown in Figure 4.14.
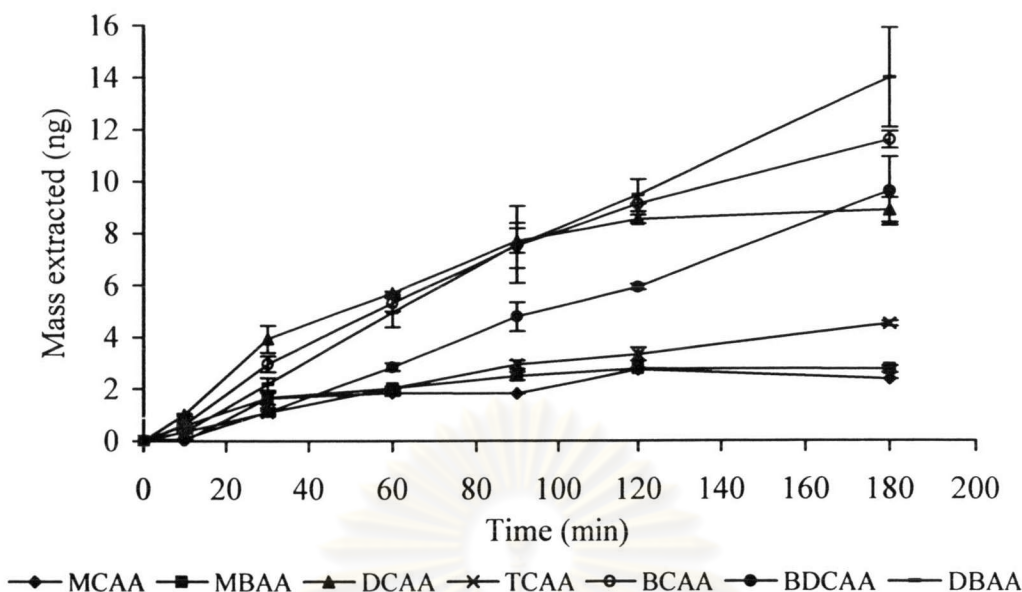
Figure 4.14 Extraction time profile of haloacetic methyl ester by in-situ methylation (MeOH: 1 mL, H_2SO_4: 1 mL, extraction temperature: 55 °C, n = 3).

It seemed that the analytical signal increased with the extraction time. The amount of the extraction was rapidly increased in initial, followed by slower increase lasting a long time. MCAA, MBAA and TCAA had reached equilibrium after 60 min, DCAA had reached equilibrium after 90 min. However, BCAA, BDCAA and DBAA had not yet reached equilibrium state after 180 min. Although equilibrations of some analytes were not attained after 180 min, however, it appeared that equilibrium was reached for most of the compounds studied after 60 min therefore the extraction time of 60 min was selected in order to avoid excessively long analysis times because quantification was possible before equilibration if the extraction conditions are constant [96]. Hence, extraction 60 min conditions were used all subsequent experiments.

4.5.5 Effect of salting out

Figure 4.15 shows the influence of Na_2SO_4 addition on the extraction efficiency of HAAs. It is evident that the addition of Na_2SO_4 from 0-20 % w/v (saturated Na_2SO_4) promotes transport of the analytes to the headspace, hence the amounts of analytes extracted were increased. This can be explained by the engagement of water molecules in the hydration spheres around the ionic salt. These hydration spheres reduce the concentration of dissolved analyte molecules in water. Hence, it is expected that this will

drive additional analytes into the headspace phase [97]. Base on the experiment, 20 % Na_2SO_4 w/v content was used for all subsequent extractions.
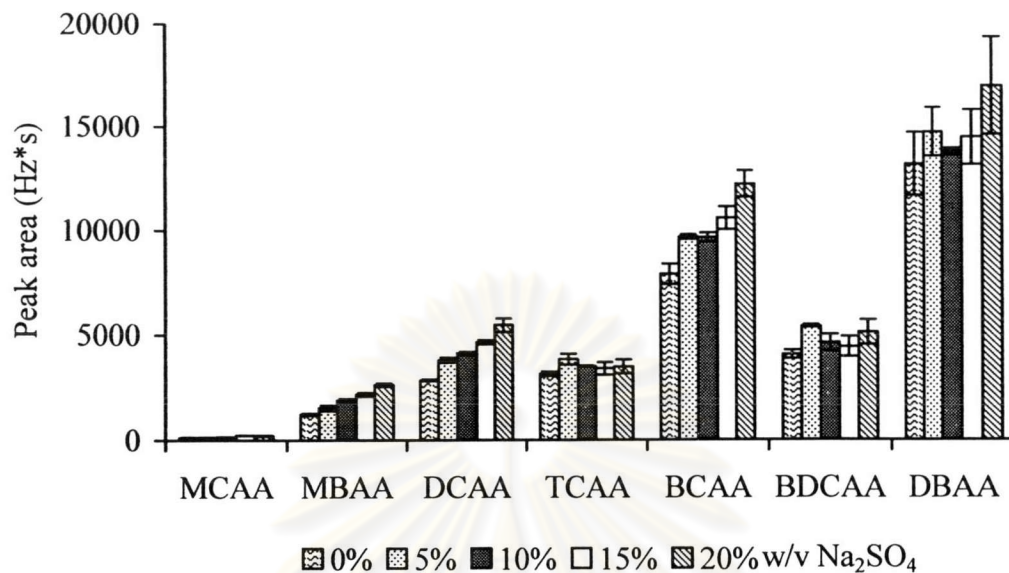

Figure 4.15 Effect of salt concentration on the extraction efficiency (MeOH: 1 mL, H_2SO_4: 1 mL, extraction time: 60 min, extraction temperature: 50 ºC, n = 3).

4.5.6 Effect of magnetic stirring

Figure 4.16 shows the influence of stirring on the extraction efficiency of HAAs. According to the statistically using the Student's *t*-test, no significant difference between non stirring and stirring were detected at the 95% confidence level (significant difference between procedures for *P*-value was 0.05) because *P*-value of all analytes are greater than 0.05 (data was shown in APPENDIX B). This may be due to that ester derivatives were high volatility, consequently after derivatized most amount of ester derivatives volatile rapidly into headspace phase. Therefore, similar responses were obtained for non stirring and stirring. Base on the experiment, non stir was used for all subsequent extractions.
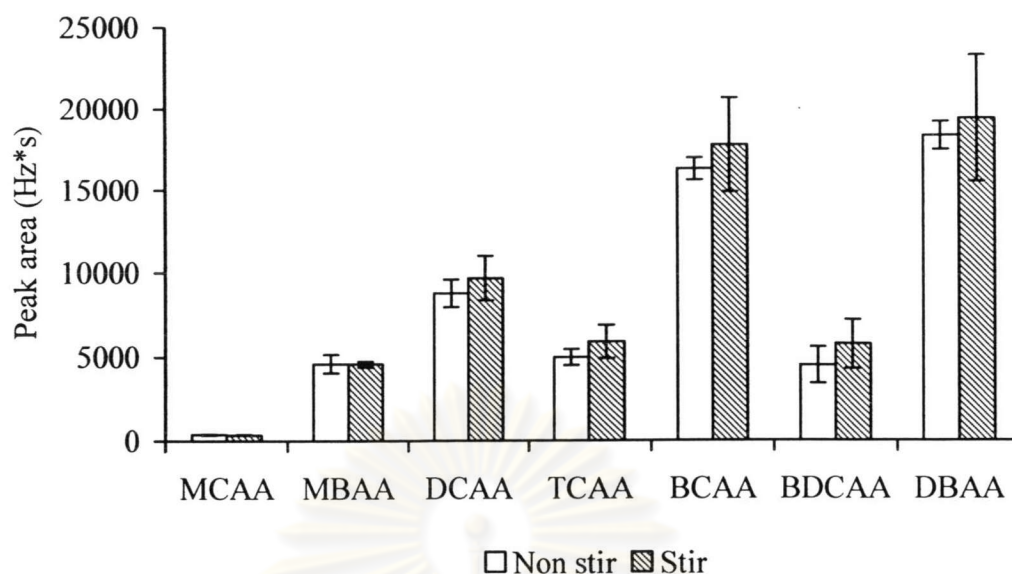
Figure 4.16 Influence of magnetic stirring on extraction (MeOH: 1 mL, H_2SO_4: 1 mL, Na_2SO_4 20 % w/v, extraction time: 60 min, extraction temperature: 55 °C, n = 3).

4.6 Method evaluation of haloacetic acids

In order to assess the performance under the above optimum condition, linearity, the method detection limit (MDL) and the method quantification limit (MQL) were evaluated. The results were shown in Table 4.7.

Table 4.7 Linear ranges, correlation coefficients (R^2) and method detection limit of liquid-phase microextraction of HAAs in water samples determined by GC-µECD.

Analyte	Linear range (µg/L)	R^2	MDL (µg/L)	MQL (µg/L)	HS method [23] MDL (µg/L)
MCAA	60-300	0.9904	18	59	-[a]
MBAA	6-80	0.9997	1	4	-[a]
DCAA	9-90	0.9940	2	8	3
TCAA	3-80	0.9967	0.4	1	0.5
BCAA	2-80	0.9973	0.2	0.6	-[a]
BDCAA	6-80	0.9904	0.9	3	-[a]
DBAA	1-80	0.9982	0.1	0.3	-[a]

[a] not reported.

The linearity were observed for the concentrations ranging from 1 to 300 µg/L with the correlation coefficients (R^2) being greater than 0.99, depending on the analytes. The MDLs of most analytes were below 1 µg/L except DCAA and MCAA that were 2 and 18 µg/L, respectively. The MDL of MCAA was high due to that it contained the lowest halogen content that yielded the poorest ECD response. Moreover, methylation efficiency of MCAA was poor with acidic methanol [98,99]. Nevertheless, the MDL of five HAAs was much lowers than the MCL values of WHO and US EPA regulation. In comparison with previously HS method that reported by Wang and Wong [23], our method exhibited lower MDLs, lower RSD and lower extraction temperature. As shown in Table 4.8, the recoveries from spiked concentration range from 5 to 90 µg/L, depending on the analytes, were 97 to 109 % with % RSD less than 12 % (the acceptable values of RSD at concentration of 90 µg/L are not greater than 23 %) [95].

Table 4.8 % Recoveries and % relative standard deviations of HAAs spiked water samples.

Analyte	Concentration (µg/L)	Recovery (%RSD), (n = 5)
MCAA	90	100 (5)[a]
MBAA	10	97 (9)
DCAA	15	97 (5)
TCAA	5	102 (6)
BCAA	10	108 (5)
BDCAA	10	103 (7)[a]
DBAA	5	109 (12)

[a] based on three replications.

4.7 The determination of THMs and HAAs in drinking water

The developed method has been applied for determination of THMs and HAAs in drinking water and tap water. The concentrations of THMs and HAAs found in drinking water and tap water were summarized in Table 4.9 and Table 4.10, respectively.

Table 4.9 THMs concentrations in drinking water from local resources and local tap water.

Sample	Concentration ($\mu g/L$)					
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	Total THMs	THMs Ratio
MilliQ water	ND	ND	ND	ND	-	-
Drinking water 1	50	7	0.4	ND	57.4	0.37
Drinking water 2	37	8	1	ND	46	0.33
Drinking water 3	ND	ND	ND	ND	-	-
Drinking water 4	ND	ND	ND	ND	-	-
Drinking water 4 + spiked THMs 10 $\mu g/L$	11	10	10	10	-	-
Drinking water 5	8	1	ND	ND	9	0.06
Mineral water 1	ND	ND	ND	ND	-	-
Mineral water 2	ND	ND	ND	ND	-	-
Tap water 1	43	9	1	ND	53	0.38
Tap water 2	47	11	2	ND	60	0.44
Tap water 3	57	12	2	ND	71	0.51
Tap water 4	58	14	2	ND	74	0.54

ND = non detected.

Table 4.10 HAAs concentrations in drinking water from local resources and local tap water.

Sample	Concentration (µg/L)							
	MCAA	MBAA	DCAA	TCAA	BCAA	BDCAA	DBAA	Sum HAA5[a]
MilliQ water	ND	ND	ND	ND	ND	ND	ND	-
Drinking water 1	ND	ND	20.3	0.6	ND	ND	ND	20.9
Drinking water 4	ND	ND	ND	ND	ND	ND	ND	-
Drinking water 5	ND	ND	4.6	ND	ND	ND	ND	4.6
Drinking water 6	ND	ND	ND	ND	ND	ND	ND	-
Mineral water 1	ND	ND	ND	ND	ND	ND	ND	-
Tap water 1	ND	ND	3.8	10.2	ND	ND	ND	14.0
Tap water 4	ND	ND	5.6	10.0	ND	ND	ND	15.6
Tap water 5	ND	0.5	11.1	3.1	ND	ND	ND	14.7

ND = non detected.

[a] Sum HAA5 refer to sum concentration of MCAA, MBAA, DCAA, TCAA and DBAA.

The range of total THMs in drinking water and tap water were ND-57.4 and 53-74 µg/L, respectively. The range of THMs ratio in drinking water and tap water were ND-0.37 and 0.38-0.54 µg/L, respectively. The concentration of chloroform was higher than other THMs and THMs concentration were generally decreased in order of $CHCl_3$ > $CHCl_2Br$ > $CHClBr_2$ > $CHBr_3$ in all sample. The range of total concentration of HAA5 in drinking water and tap water were ND-20.9 and 14.0-15.6 µg/L, respectively. The quality control samples from spiked matrix with THM 10 µg/L (spiked drinking water 4 in Table 4.9) showed that extraction efficiency of the method were very good. Low cost drinking water tended to show higher concentration of THMs and HAAs, which may suggest that these water be produced from tap water, whereas drinking water produced from ground water and mineral water showed lower concentration of THMs and HAAs, which may be due to that these water contained small amount of organic matters. However, the concentrations of THMs ratio and total HAA5 in drinking water and tap water samples in this survey were below the guided values of WHO, which are a standards of Thailand. Moreover, there were lower than the MCL of US EPA and EU.