4 DISCUSSION AND CONCLUSIONS

4.1 Discussion

Determination of PCBs in milk and water samples in this work was attempted, using C_{18} SPE cleanup method prior to the analysis by GC/ECD .

In the milk sample analysis, both the temperature programs and detection limits of GC/ECD with HP608 column were investigated. The cleanup and extraction were performed on a C_{18} sorbent of which the volume of water added, conditioning effect, drying effect and elution volume were studied. The optimum conditions were used on the subsequent percent recovery study of spiked cow's milk and powdered milk samples at two spiked levels.

In the water sample analysis, both the temperature programs and detection limits of GC/ECD with DB-1701 column were investigated. The cleanup and extraction were performed on a C_{18} sorbent of which the type and amount of wetting solvents and sample volume were studied. The optimum conditions were used on the subsequent percent recovery study of spiked drinking and natural surface water samples. The analysis of real samples was performed on three brands of drinking water and four sources of natural surface water from the northern part of Thailand.

In this work, among 209 PCB congeners, only 7 congeners were studied, namely PCBs No. 10, 28, 52, 101, 138, 153 and 180. PCB No. 10 was selected to test the efficiency of the developed method in the analysis of low chlorinated PCBs. The rest of PCBs were selected according to the German guidelines.[1] In the early stage of PCBs analysis, the commercial mixtures of PCBs were selected as the standards. However, they were found to be not suitable. It should be noted that PCBs in the environment differ from the commercial mixtures.[106]

Only some congeners, namely PCBs No. 28, 52, 101, 138, 153 and 180, were analyzed,. These congeners were selected due to their distribution throughout the chromatogram, coverage of the chlorinated range, use in technical mixtures, relative ease of analytical determination, and proved toxicity. Besides, they are commonly referred to in environmental and food regulations.[33]

According to the EPA method 608 which is usually used in the determination of PCBs in waste water, the GC temperature program of this method was modified for determination of PCBs in milk sample. In this EPA method, HP 608 capillary column was used with the following temperature program 80°C - 80°C (1 min) - 190°C (30°C min^{-1}) - 280°C (6°C min^{-1}) - 280°C (1 min) - 300°C (20°C min^{-1}) - 300°C (5 min). The chromatogram of PCBs from this temperature program is shown in Figure 3.1.

In Figure 3.1 the retention time of the last eluted compound was 17.29 min which was satisfactory. However, peaks 1, 2 and 3 were observed to be affected by some interferences in the region of retention time 5-11 min, resulting in rather broad peaks with lower peak areas and peak heights than what were obtained with other methods, as shown in Tables 3.2 and 3.3 and Figures 3.7 and 3.8. Therefore, it was necessary to optimize this GC temperature program. Methods A-E were thus obtained by a trial and error approach. In the GC temperature program methods A-E, the retention time of the last eluted compound was in the same range, 16.9-18.7 min (Table 3.1). But these methods gave different detector responses, peak heights and peak areas for most of the studied PCBs, as shown in Table 3.2 and Figure 3.7 for peak area comparison and Table 3.3 and Figure 3.8 for peak height comparison. Methods A-E gave the same range of peak area and peak height for PCB No. 10. For PCBs No. 28 and 52 they could not be separated in the GC temperature program method A, and tend to increase in peak area and peak height from the GC temperature program methods B to E. PCB No. 101 tended to increase in peak area from the GC temperature program method A to C and gave the same range of peak area in the GC temperature program methods D and E. PCBs No. 138 - 180 tended to increase in peak area from the GC temperature program methods A to B and gave the same ranges of peak area as in the GC temperature program methods C to E. In the peak height (Table 3.2 and Figure 3.8), PCBs No. 101 - 180 tended to increase in peak height from the GC temperature program methods A to E and gave the maximum peak height in the GC temperature program method E. From the peak area, peak height and peak shape of the PCBs in each temperature program method, temperature program method E gave the best results in both response and peak shape, as shown in Tables 3.2 and 3.3 and Figures 3.6-3.8. Thus, this temperature program was used in the milk sample analysis.

In an attempt to explain the broad peak phenomenon, especially of peak No. 2 (PCB No. 28), and low response of some PCBs in the GC temperature program methods EPA 608, A, B and C and improving of broad peaks in the GC temperature program methods D and E, details of these GC temperature programs are shown as follows :

EPA 608 : 80°C - 80°C (1 min) - 190°C (30°C min^{-1}) - 280°C (6°C min^{-1}) - 280° C (1min) - 300°C (20°C min^{-1}) - 300°C (5 min)

Method A : 150°C - 150°C (1 min) - 280°C (7°C min^{-1}) - 280°C (7min)

Method B : 160°C - 160°C (1 min) 270°C (6°C min^{-1}) - 280°C (20°C min^{-1}) - 280°C (7 min)

Method C : 170°C - 170°C (1 min) - 265°C (5 min^{-1}) - 280°C (20°C min^{-1}) - 280° C (7min)

Method D : 180°C - 180°C (1 min) - 215°C (4°C min^{-1}) - 275°C (6°C min^{-1}) - 280°C (20°C min^{-1}) - 280°C (7 min)

Method E : 190°C - 190°C (4 min) - 215°C (5°C min^{-1}) - 280°C (6°C min^{-1}) - 280°C (7min)

From the GC temperature program methods above, they differed in the temperature program rate and the initial temperature setting. However, the temperature program rate did not differ significantly and thus should not be used to explain the broad peak phenomenon and low response results. The broad peak could possibly result from an interaction between active sites in the chromatographic system and analyte molecules.[102] Increasing of the initial temperature may circumvent the interaction between active sites and analyte molecules thus the sharper peaks and higher detector response was obtained in using the GC temperature program methods D and E. The optimized GC conditions are summarized and shown in Table 3.5.

PCB No. 28 was found to be the broadest peak. This phenomenon can be explained from its structure. PCB No. 28 is the only one congeners in this work which contains only one ortho chlorine substitution while the others contain two ortho chlorines substitution (see Appendix A). The high degree of substitution at ortho position favors a more perpendicular, thus less planar arrangement between two phenyl rings.[107,108] The less planarity led to less contact with the stationary phase[2] and consequently the active sites too. Therefore the congeners with less planarity

(PCBs No. 10, 52, 101, 138, 153 and 180), all have two ortho chlorine in the molecule and are prone to give sharper peaks. But the PCB No. 28 which has one ortho chlorine atom, indicating a higher degree of planarity than the others, should be in a position to have more contact with the active sites, resulting in the broad peak phenomenon.

The elution order (retention time) of PCBs obtained with the HP-608 capillary column was PCBs No. 10 < 28 < 52 < 101 < 153 < 138 < 180. The PCBs No. 10, 28, 52, 101, 153,138 and 180 are di-, tri-, tetra-, penta-, hexa-, -hexa and hepta-chlorobiphenyl, respectively. Thus, elution order could be explained in terms of boiling point, degree of chlorination and polarizability. A higher degree of chlorination increases the boiling point and therefore the retention time sequence was found to be di- < tri < tetra < penta < hexa < hepta-chlorobiphenyl, respectively.[2] In the case of PCBs No. 138 and 153 in which both are hexachlorobiphenyls, the elution order could be explained in terms of polarizability. PCBs No. 138 and 153 are 2,2′,3,4,4′,5′- and 2,2′,4,4′,5,5′-hexachlorobiphenyl, respectively. The adjacent chlorine atoms could increase the net molecular polarizability.[108] Therefore the chlorine atoms at positions 2, 3 and 4 of PCB No. 138 can induce more polarizability than in PCB No. 153. Thus PCB No. 138 was eluted after PCB No. 153.

The limit of detection in milk sample analysis was calculated based on a method described by Miller and Miller.[104] This method is based on utilization of an external calibration curve and its corresponding y-intercept (see Appendix B for more detail). In this work, the calibration curve was constructed from peak areas and corresponding PCBs concentrations in the range of 0.5 –7 $\mu g\ l^{-1}$. The calibration curves of PCBs No 10, 28, 52, 101, 138, 153 and 180 are shown in Figures 3.9, 3.10, 3.11, 3.12, 3.13, 3.14 and 3.15, respectively. The linearity of these calibration curves is indicated by the value of r^2. The r^2 values for PCBs No. 10, 28, 52, 101, 138, 153 and 180 are 0.9980, 0.9994, 0.9930, 0.9952, 0.9953, 0.9989 and 0.9963, respectively. The limits of detection of PCBs No. 10, 28, 52, 101, 138, 153 and 180 are 0.42, 0.22, 0.79, 0.90, 0.65, 0.31 and 0.76 $\mu g\ l^{-1}$, respectively, as shown in Table 3.4.

For determination of organohalogen compounds, including PCBs in fatty matrices samples, it is normally based on total fat extraction with non polar organic solvents and cleanup with normal phase adsorbent,[48,109-111] normal phase coupled

with reverse phase adsorbents,[31,112] chemical decomposition (H_2SO_4 or saponification)[113,114] or GPC.[13,115,116] But the total fat extraction approach posed some problems such as consumption of large amount of solvents and sorbents and formation of emulsion. Besides, extensive cleanup[117] was required. Thus a new approach was attempted. This approach utilized polar organic solvents or nonpolar organic solvents added with the polar one to extract organohalogen compounds from the fatty sample matrices with minimum fat extraction. Therefore, these extraction methods reduced the cleanup process[34]. Many organic solvent systems were proposed such as petroleum ether-acetonitrile-ethanol (for milk samples),[117] methanol-water[34,118,119] (for milk samples), acetonitrile(for eggs),[55] petroleum ether-acetonitrile (for poultry fat),[120] hexane-acetonitrile-ethanol(for human milk),[121] ethylacetate-methanol-acetone(for milk)[105] and hexane-acetonitrile-ethanol(for milk).[122] After extraction, they were normally cleaned up with normal phase sorbents[117,120-122] or coupled between normal phase and reversed phase sorbents.[55] Only some workers used a reversed phase sorbent alone to clean up the extraction. Picó *et al.* used water and methanol mixed with milk and loaded onto C_{18} SPE.[34,118,119] Prapamontol and Stevenson used ethylacetate-acetone-methanol to extract organochlorine from milk and cleaned up with C_{18} SPE.[105] The advantages of reversed phase SPE are that the amount of solid phase and organic solvent used and analysis time are reduced[34] and the potential to reuse.[63] Therefore, in this work, milk samples were extracted with ethylacetate-methanol-acetone system and cleaned up with C_{18} SPE. First of all, the SPE was optimized by using PCBs spiked water sample (instead of milk samples) with the solvent system, ethylacetate : acetone : methanol (1 :2 : 2 v/v). PCBs spiked water sample was used instead of milk samples here in order to check both the efficiency of C_{18} SPE in retaining PCBs and the efficiency of the solvent system in extraction of PCBs from the milk samples. In this work, the flow rate through the sorbent and the effect of adding salt were not investigated since these they have been reported to have no effect on extraction.[119] PCBs are hydrophobic compounds thus increasing the polarity of the solvent system should improve the extraction efficiency.[50] Therefore, water with a volume of 10, 20 and 30 ml was added into the spiked solutions which contained 50 ng PCBs. The spiked solutions were passed through C_{18} SPE which had not been conditioned with solvent mixtures of water:

ethylacetate: acetone: methanol and eluted with isooctane to determine the percent recoveries. The percent recoveries are shown in Table 3.6. For PCB No. 10, a volume of 10 and 30 ml of added water gave lower than 50% recovery and 51% recovery when 20 ml of water was added. For PCBs No. 28 and 52, adding 10, 20 and 30 ml of water gave more or less the same of percent recoveries. For PCBs No. 101- 180, adding 10, 20 and 30 ml of water gave the percent recovery closed to 100%. Therefore, from the results obtained, it could be noted that increasing the amount or volume of water added did not improve the percent recoveries significantly.

Effects of conditioning the C_{18} with the solvent mixtures which were used to prepare solutions A, B and C were investigated. C_{18} SPE was conditioned with dichloromethane, then methanol and finally with the solvent mixtures which were used to prepare solutions A, B and C. Then the solutions A, B and C were loaded onto each cartridge. The conditioning was used to ensure a good contract between analyte and sorbent in the adsorption step.[50] The percent recoveries are shown in Table 3.7. Conditioning with solvent mixtures of solution A did not improve the percent recoveries compared with without conditioning. Conditioning with solvent mixtures of solution C improved the percent recoveries of PCBs No. 10, 28 and 52 but decreased in PCBs No. 101-180 compared with without conditioning. Conditioning with solution B improved the percent recoveries of PCBs No. 10 and 28 and gave the same recoveries for the rest of PCBs compared with without conditioning and the precision (%R. S. D.) was improved significantly in PCBs No. 10 and 28. Therefore, conditioning is necessary for C_{18} SPE in detection of PCBs. The conditioning with solvent mixture of solution B was selected for the next experiment. As shown in Tables 3.6 and 3.7, the percent recovery of PCB No. 10 is lower than the rest significantly. This might have been due to the high vapor pressure of dichlorinated biphenyl (PCB No. 10), 0.24 Pa at 25°C.[2] Therefore, PCB No. 10 was likely to lose its original amount during the drying step which took approximately 30 min. Effects of the drying step on percent recoveries of PCBs was thus investigated. After conditioning the C_{18} SPE, 100 µl of 500 µg l^{-1} PCBs in isooctane was injected onto the top of sorbent and dried for 30 min. Then they were eluted with isooctane and determined the percent recoveries. The results are shown in Table 3.8. The recoveries of all PCBs were found to be closed to 100% and thus no loss during

drying the cartridge could be assumed. The lower percent recoveries of PCB No. 10 may be due to its higher polarity than the other PCBs. Thus PCB No. 10 could not be retained well in C_{18} SPE. The last step in optimization of C_{18} SPE was an investigation on the elution volume. The aim of this investigation was using minimum amount of elution solvent (isooctane). The minimum amount of elution solvent was required to avoid interference from co-eluted substances[50] and the need for a concentration step later in the procedure. In this work, isooctane was used as eluting solvent due to its ability to dissolve PCBs and reasonably high boiling point which minimized the change in concentration during storage. The spiked solution was loaded onto C_{18} SPE and each fraction (1 ml) of the eluent were collected and subjected to GC/ECD. Peak area in each fraction was determined as shown in Table 3.9. In this work, a gravity flow rate was used in the elution step to in order to ensure the use of minimum volume. All of PCBs were eluted in fraction 2. Hence 2 ml of isooctane was established as a suitable volume for the eluent. After optimization process, the optimum conditions were obtained and are shown in Table 3.10.

The C_{18} SPE was first conditioned with dichloromethane, methanol and solvent mixtures (water : ethylacetate : acetone : methanol, 10 : 1 : 2 : 2 v/v) with a flow rate of about 1-2 ml min^{-1}. Then the sample solution was loaded with a flow rate of about 2-3 ml min^{-1} and dried for 30 min. Finally the analytes were eluted with 2 ml of isooctane with a gravity flow rate. The optimum condition of SPE was brought to milk sample extraction. In the optimization of milk sample extraction, spiked cow's milk at 10 $\mu g\ l^{-1}$ was investigated. The spiked cow's milk was extracted with ethyl acetate : acetone : methanol and the supernatant was mixed with water and loaded onto C_{18} SPE. The extraction system for 2 ml of spiked cow's milk was 2 ml ethyl acetate, 4 ml methanol, and 4 ml acetone and the volume of water added was 18 ml, according to the results obtained from the attempted optimization in SPE. As in previous reports,[119,122] methanol or ethanol could destroy the fat globules and thus improve the extraction of PCBs from trapping but methanol has been reported to be better in penetrating the fat globule due to the low viscosity.[119] Therefore, methanol was used here.

Acetone was used to assist in protein precipitation.[119] There is no clear explanation as to why ethylacetate is often used as an extractant apart from the fact

that its low polarity permits dissolution of PCBs with a low amount of fat which is then coextracted. Ethylacetate is miscible with acetone and methanol and can be partially dissolved in water thus no emulsion occurs. In the extraction, ultrasonication was used to assist in breaking down of milk fat globules.[105] After ultrasonication, the supernatant was separated from milk residue with the aid of centrifugation. Then the supernatant was mixed with water to increase the polarity of the solution system and loaded onto C_{18} SPE. The results of a single extraction are shown in Table 3.11. From this table, low recoveries of all PCBs were obtained. This might be attributed to PCBs remaining in milk residue due to some attraction. Thus, the second extraction was performed. After the first extraction, water and the extraction solvent (ethyl acetate : methanol : acetone) were added to the milk residue and extraction in the same manner as in the first extraction was performed. The supernatant was pooled with water added and then loaded onto C_{18} SPE. The percent recoveries of all PCBs were improved in the double extraction method. This means that the first extraction was not adequate in extracting all of PCBs from the milk sample and that the second extraction was necessary here. The percent recoveries of PCBs in the double extraction in the range of 63-100 % with % R.S.D. lower than 7 % are shown in Table 3.11 . In the analysis of powdered milk, double extraction was therefore performed. The lower percent recovery of PCB No. 10 may be due to its higher polarity than the others as already explained in the discussion on the SPE above. The cow's milk was used as the blank sample and extracted in the same manner as that for the spiked sample. The chromatograms of blank and spiked cow's milk for double extraction are shown in Figures 3.16 and 3.17, respectively. The efficiency of this method in removing the matrices can be seen in Figure 3.16 which shows a clean chromatogram of non-spiked cow's milk. The extraction method was also performed for the powdered milk which was reconstituted in water and spiked with PCBs. Then it was extracted in the same manner as in the procedure for cow's milk. In the case of powdered milk spiked at 74.1 $\mu g\ kg^{-1}$, PCB No. 10 could not be detected, whilst PCBs No. 28, 52 and 101 yielded lower than 50% recoveries and PCBs No. 138- 180 gave 67-77% recoveries with the % R. S. D. in the range of 6.4-7.3%. The chromatogram of spiked powdered milk at 74.1 $\mu g\ kg^{-1}$ is shown in Figure 3.21. The non-spiked powdered milk was used as the blank solution and was extracted in the same manner

as spiked powdered milk and the resultant chromatogram is shown in Figure 3.20. In Figure 3.20 a clean chromatogram was obtained, indicating the efficiency of the extraction method in removing the matrices. The lower percent recoveries of spiked powdered milk at 74.1 μg kg^{-1} were comparable those of the spiked cows milk at 10 μg l^{-1} (the same amount of spiked PCBs) which could be explained in terms of organochlorine compounds being entrapped in the denatured protein of the powdered milk.[123] It was observable that during the second extraction the milk residue appeared to be tightly packed even during ultrasonication which prevented the extraction solvents to penetrate into the traps. According to the limit value set in Germany in 1988, PCBs limits in milk were 40 μg kg^{-1} for congeners 28, 52 and 101 and 180, 50 μg kg^{-1} milk fat for congeners 138 and 153. Therefore the fat content in milk was determined with hexane: acetone extraction.[105] The fat content in cow's milk was found to be 3.47% (w/v) and in that in powdered milk was 25.76% (w/v), as shown in Table 3.12. To test validity of the proposed method, PCBs were spiked into the cow's and powdered milk at the German limit set, i.e. 40 μg kg^{-1} milk fat. Cow's and powdered milk samples were thus spiked at 1.4 μg l^{-1} and 10.4 μg kg^{-1}, respectively. The spiked cow's milk at 1.4 μg l^{-1} and the blank sample were extracted in the same manner as in the spiked cow's milk at 10 μg l^{-1} except the final volume was reduced to 1 ml. The percent recoveries are shown in Table 3.13. PCB No. 10 could not be detected. The percent recovery of PCB No. 28 was lower than 36%. The percent recovery of PCB No. 52 was 36%. As for PCBs No. 101- 180, the percent recoveries were in the range of 53-64% with the % R. S. D. in the range of 2.8-11.2%. The chromatograms of non-spiked and spiked cow's milk at 1.4 μg kg^{-1} are shown in Figures 3.18 and 3.19, respectively. In Figure 3.19, peaks of PCBs No. 101-180 are clearly more eminent than those of PCBs No. 28 and 52. In Figure 3.18, the non-spiked cow's milk obtained with the final volume reduced to 1 ml shows a fairly clean chromatogram. The percent recoveries of all PCBs spiked at 1.4 μg l^{-1} were found to be lower than those spiked at 10 μg l^{-1} spiked level. This may be due to the loss of PCBs during the extraction step. The spiked powdered milk at 10.4 μg kg^{-1} and the blank sample were extracted in the same manner as spiked powdered milk at 74.1 μg kg^{-1} except the final volume was reduced to 1 ml. The percent recoveries are shown in Table 3.13. PCB No. 10 was found to be undetected. The percent recoveries of

PCBs No. 28 and 52 were lower than 36%. The percent recoveries of PCBs No. 101-180 were in the range of 57-65% with the % R.S.D. in the range of 3.7-10.5%. The chromatograms of blank sample and spiked powdered milk are shown in Figures 3.22 and 3.23, respectively. In Figure 3.22, a clean chromatogram was obtained. In Figure 3.23, peaks of PCBs No. 101-180 are clearly seen while those of PCBs No. 28 and 52 were found to be less eminent. From the data in Table 3.13, the extraction of PCBs from the powdered milk with this proposed method yielded lower efficiency than the extraction procedure for cow's milk describe above. Therefore, a more penetrating solvent system was desirable.

Although this proposed method gave low recoveries at the German regulation limit of the spiked levels in both types of milk but some advantages can be envisaged, namely (1) efficiency in cleaning up the matrices, (2) low amount of samples required (2 ml), resulting in a need for only small set of glassware and extraction equipment, and (3) possibility for further development, using an appropriate internal standard. Regarding the last advantage, a higher pre-concentration factor should be investigated and also the cartridge reusing. This method should then be improved in terms of efficiency in order to determine PCBs in milk samples with a lower cost, higher speed and less waste produced.

In the water sample analysis, the GC capillary column was changed to DB-1701, (14%-Cyanopropyl-phenyl)-methyl polysiloxane[102] which should provide high resolution due to the more polarity of the stationary phase and small internal diameter. The temperature programs were optimized, obtaining methods F, G, H and I, as described in Section 3.2.1. All of these methods gave the same range of peak areas and peak heights and there was no need to show such data here. The retention time (t_R) and retention time difference (Δt_R) were used as guiding parameters to choose a suitable temperature program. The relevant retention data are shown in Table 3.14. The temperature program methods F and H gave a longer retention time than methods G and I. Thus the last two methods were selected. In considering adjacent peaks (PCBs No. 28 and 52, PCBs No. 138 and 153, PCBs No. 138 and 180), the Δt_R of each of two adjacent peaks was calculated and the data obtained were used in the selection of a suitable GC temperature program. In the temperature program method G, Δt_R values for PCBs No. 28 and 52, 138 and 153 and 138 and 180 were found to be

0.71, 1.86 and 4.26 min., respectively. In the temperature program method I, Δt_R values for PCBs No. 28 and 52, 138 and 153, and 138 and 180 were 1.05, 1.24 and 2.92 min, respectively. In the analysis of water samples, in the region of PCBs No. 28 and 52 peaks, interference was observable and thus increasing the Δt_R value between PCBs No. 28 and 52 should decrease the probability of PCB No. 28 overlapping with PCB No. 52 and some interference peaks. Thus the temperature program method I was selected. The optimal GC conditions with DB-1701 column are summarized in Table 3.16. The chromatogram obtained from using the temperature program method I is shown in Figure 3.24 which illustrate symmetrical and sharp peaks. The elution order of all PCBs was found to be the same as that found when an HP-608 column was used and an explanation of this elution order is similar what is already explained above. The limit of detection in the water sample analysis was calculated based on a method described by Miller and Miller.[104] This method is based on an external calibration curve and the associated y-intercept (see Appendix B for more detail). The calibration curve was constructed from peak areas and corresponding PCBs concentrations in the range of 0.1-1.5 $\mu g\ l^{-1}$. The calibration curves of PCBs No. 10, 28, 52, 101, 138, 153 and 180 are shown in Figures 3.25, 3.26, 3.27, 3.28, 3.29, 3.30 and 3.31, respectively. These calibration curves are in good linearity, as indicated by high values of r^2. The r^2 values of PCBs No. 10, 28, 52, 101, 138, 153 and 180 were found to be 0.9989, 0.9930, 0.9954, 0.9893, 0.9934, 0.9935 and 0.9918, respectively. The limits of detection of PCBs No. 10, 28, 52, 101, 138, 153 and 180 are 0.06, 0.14, 0.12, 0.18, 0.16, 0.14 and 0.15 $\mu g\ l^{-1}$, respectively, as shown in Table 3.15. PCBs in water samples are usually of very low concentration due to their hydrophobic property. Thus they tend to accumulate on organic particles rather than dissolve in water. The pre-concentration was necessary in this work. C_{18} SPE was selected as a pre-concentration means due to its advantages such as reduced amount of organic solvents, analysis time and cost in analysis. Additionally, this pre-concentration with C_{18} SPE should offer rapidity in the analysis, minimal use of glassware and a high pre-concentration factor. Effects of the flow rate, pH and addition of salt were not attempted here as they have been claimed to have no effect in extraction of non-ionized molecules in water.[27,124,125] An investigation in the C_{18} SPE optimization was performed, using 0.1 $\mu g\ l^{-1}$ of PCBs spiked in distilled water with

various solution compositions as shown in Table 3.17. In the determination of PCBs or organochlorine compounds in water, some workers prefer to load the water sample directly onto C_{18} SPE[27] or use a small amount of organic solvent as wetting solvent.[23,126] But in this work, the same procedure, i.e. loading without organic solvent, was tried out and the percent recoveries are shown in Table 3.18. In the solution D in which no organic solvent was added, the percent recoveries were lower than 50% for all of the studied PCBs. Therefore, the type and amount of organic solvent added were investigated. The wetting solvent was used to maintain the hydrophobic property of C_{18} chain.[126] In the solutions E, F and G, 50 ml of water was used and 5 ml of acetone, methanol and isopropanol were added, respectively. The percent recoveries of solutions E, F and G are shown in Table 3.18. Using acetone as wetting solvent (solution E), the percent recoveries of PCBs No. 28, 52 and 101 were improved compared with solution D. Using methanol as wetting solvent (solution F), the percent recoveries of PCBs No. 28 - 180 were higher than 58% but the percent recovery of PCB No. 10 was still lower than 50%. Using isopropanol as wetting solvent (solution G), the percent recoveries of PCBs No. 28-153 were higher than 50% but the recovery of PCB No. 180 was still lower than 50%. From this investigation, methanol and isopropanol were seen to be better wetting solvents than the rest but isopropanol is a more expensive solvent and gave a rather low percent recovery for PCB No. 180 which is a regulated PCBs. Methanol was thus selected for further investigation. Investigation on increasing amount of organic solvent was performed with solutions H and I. In the solution H, 20 ml of methanol was used as wetting solvent. The percent recoveries of PCBs in solution H are shown in Table 3.19. The percent recoveries of PCBs No. 52-180 were improved compared with the values obtained from solution F and the percent recoveries were above 77% with the % R.S.D. in the range of 3.7-22.5 %. In the solution H, PCB No. 10 could not be detected. This may be due to high polarity of PCB No. 10, high amount of organic solvent in the sample solution and high amount of total sample volume (70 ml). PCB No. 10 could possibly dissolve better in the eluent and could not be retained by C_{18}. The percent recoveries of all studied PCBs in solution H were not all that satisfactory and thus another organic solvent system, solution I, was attempted. In the solution I, 50 ml of water was added with 4, 8 and 8 ml of ethylacetate, methanol and acetone,

respectively. The percent recoveries of PCBs in solution I are shown in Table 3.19. PCB No. 10 was still not detected. An explanation for this is the same as that accountable for solution H. But the percent recoveries of PCBs No. 28-180 were improved compared with all previous optimized solutions. They were in the range of 72-94% with the % R.S.D. in the range of 1.2-12.5 %. Some improvement should still be performed to recover PCB No. 10. The PCB No. 10 is the most polar PCBs in this work. It yielded a low break through volume in C_{18}. Therefore reduction in the sample solution volume should retain this PCBs more effectively. For the solutions J and K, the spiked distilled water was reduced to 20 ml. 10 ml methanol was used as wetting solvent in solution J. The percent recovery data are shown in Table 3.20. The percent recoveries of all studied PCBs were in the range of 57-92 % with % R.S.D. in the range 0.5-21.4 %. The percent recoveries of PCB No. 10 was improved, but with high % R.S.D. The recovery value for PCB No. 28 was improved significantly. Recoveries for PCBs No. 52 and 101 were also improved but to a lesser extent. Recovery values for PCBs No. 138, 153 and 180 were worse than those obtained with solution H. In the solution K, 10 ml of solvent mixture (ethylacetate : acetone : methanol, 1 : 2 : 2 v/v) was mixed with 20 ml spiked water. The percent recoveries of solution K are shown in Table 3.20. The percent recoveries of all studied PCBs were found to be in the range of 70-95 % with % R.S.D. in the range of 5.2-11.9 %. The percent recoveries of PCBs in solution K were compared with those obtained with the solution I. PCB No. 10 was detectable here with a percent recovery of around 70%. Recovery data for PCBs No. 28, 52, 101 and 180 were seen to be improved but those for PCBs No. 138 and 153 were not better. Therefore, in an attempt to recover PCB No. 10, the total volume of sample solution should be reduced. Between solutions J and K, the solution J gave a higher percent recovery in all studied PCBs. Hence 20 ml of water sample and 10 ml of ethylacetate : acetone : methanol (1 : 2 : 2 v/v)as wetting solvent were used. The optimal extraction conditions in the water sample analysis are summarized in Table 3.21.

Yang *et al.*[29] and Langenfeld *et al.*[127] claimed that unsilanized glassware could adsorb the nonpolar organic compounds in the water sample. This phenomenon can be used to explain what was found in this work. In the solution D in which no wetting solvent was added, PCBs tended to adsorb on the wall of the glassware yielding a low

percent recovery. In adding organic solvent to the water in the solutions E to G, the percent recovery was improved which could be attributed to PCBs being dissolved more to the solution and thus retaining on the C_{18} sorbent. More improvement in the percent recoveries were obtained when more organic solvent was added to the solutions H and I. But the solution H and I had an early break through volume which resulted in a low percent recovery for PCB No. 10.

The optimum conditions from Table 3.21 were brought to use in the analysis with real drinking and natural surface water samples and spiked water samples (drinking and natural surface water). The drinking water brand A was spiked at 0.1 μ g l^{-1} of all studied PCBs according to the EU regulation.[4] The percent recovery data are shown in Table 3.22. The chromatogram is shown in Figure 3.32. The percent recoveries of all studied PCBs except PCB No. 10 are higher than 79% with the % R.S.D. lower than 9.5%. The percent recoveries of PCBs No. 10, 28, 52, 153 and 180 in spiked drinking water were lower than in spiked distilled water (solution K) and those of PCBs No. 101 and 138 were in the same range.

A Mae-ngud natural surface water sample was spiked at 1.0 μg l^{-1} of all studied PCBs according to the EU regulation.[4] The percent recoveries data are shown in Table 3.23. The chromatogram is shown in Figure 3.33. The percent recoveries of all studied PCBs except PCB No. 10 were higher than 63 % with % R.S.D. in the range of 6.0-14.5 %. The percent recoveries of all studied PCBs were found to be lower than what was obtained with spiked distilled water. This may be due to the presence of dissolved organic compounds in water assisting in dissolving of PCBs.[128] Thus they could not be retained well in C_{18} SPE.

The proposed extraction method for PCBs in water gave fairy good results. It can be used as the screening method, providing rapidity of analysis, low cost and small sample amount requirement. The internal standard should be used to compensate for the loss during extraction.

In the analysis of water samples in this study, three brands of drinking water and four sources of natural surface water from northern of Thailand were analyzed. The chromatograms obtained from drinking water brands A, B and C are shown in Figures 3.34, 3.35 and 3.36, respectively. All of the studied PCBs were not detected in all three brands of the drinking water. As for the natural surface water, samples were

taken from Mae-ngud dam, Mae-Kuang river, Hauyhong-Krai reservoir, and Mae-ping river. All of these samples were filtered, using 0.45 μm cellulose acetate to provide only dissolved PCBs, if present. The chromatograms obtained from Mae-ngud dam, Mae-Kuang river, Hauyhong-Krai reservoir, and Mae-ping river are shown in Figures 3.37, 3.38, 3.39 and 3.40, respectively. All of the studied PCBs were not found in water samples from Mae-ngud, Mae-Kuang and Hauyhong-Krai. But the water sample from Mae-Ping yielded a chromatogram showing some matrix interference, as shown in Figure 3.40. The chromatographic pattern is similar to that of a chromatogram obtained from sulfur-containing sediment, as shown in Figure 4.1.

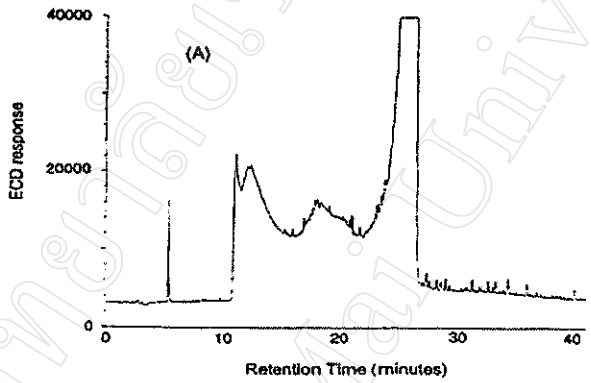

Figure 4.1 GC/ECD chromatogram of 1 g sulfur–containing sediment by SFE with pure CO_2.[16]

The matrix interference in the Mae-Ping water sample could possibly arisen from sulfur compounds. The GC/MS was used to clarify this assumption. A suitable amount of the extractant of Mae-Ping water sample was injected into the GC/MS and the chromatogram obtained is shown in Figure 3.41. On the chromatogram, there are 2 peaks at scan No. 516 and 941. The mass spectra from scan No. 516 and 941 are shown in Figures 3.42 and 3.43, respectively. The standard sulfur was also injected into GC/MS under the same operating condition and the chromatogram obtained is shown in Figure 3.44. There are 2 peaks at scan No. 516 and 942 and the corresponding mass spectra are shown in Figures 3.45 and 3.46, respectively. In Figure 3.46, it is the mass spectrum of S_8 (mw = 256) which shows the molecular ion at m/z 256 and characteristic fragments at m/z = 224, 192, 160, 128, 96 and 64, associating with the loss of 1, 2, 3, 4. 5, and 6 sulfur atoms, respectively. In Figure 3.45, it is the mass spectrum of S_6 (mw = 192) which shows the molecular ion at m/z

192 and fragments at m/z 160, 128, 96 and 64, indicating the loss of 1, 2, 3, and 4 sulfur atoms, respectively. In considering the similarity in both chromatogram and mass spectrum patterns of Mae-Ping sample and standard sulfur, it can be concluded that the interference matrices in Mae-Ping sample were sulfur molecules. To remove them, copper or tetrabutyl ammonium sulfite (TBA) should be used.[16,20,129]

4.2 Conclusions

Milk and water samples were extracted with C_{18} SPE and quantification of the PCBs in the samples analyzed were carried out using GC/ECD.

In the milk sample analysis, PCBs were spiked into distilled water and the solution was used as a model for optimization in C_{18} SPE. The results of optimization indicated that using 20 ml water and conditioning the C_{18} with solvent mixtures of water : ethylacetate : acetone : methanol (10 : 1 : 2 : 2 v/v) could improve the percent recoveries of some PCBs. The drying step had no effect on the percent recoveries of all PCBs. The elution was performed by using 2 ml of isooctane. The optimized conditions were used with spiked and non-spiked cow's and reconstituted powdered milk as follows : Milk samples were extracted two times with the solvent mixture of ethylacetate : acetone : methanol (1 : 2 : 2 v/v). The supernatant was mixed with water and loaded onto the 500 mg of C_{18} SPE . The C_{18} SPE was conditioned with dichloromethane, methanol and solvent mixtures of water : ethylacetate : acetone : methanol (10 : 1 : 2 : 2 v/v). Water was used as the wash solvent with isooctane as the eluent. The quantification was performed on GC/ECD with an HP-608 capillary column. The temperature program was 190°C - 190°C (4 min) - 215°C (5°C min^{-1}) - 280°C (6°C min^{-1}) - 280°C (7 min). The detection limit was in the range of 0.22–0.90 $\mu g\ l^{-1}$ for the studied PCBs.

In the water sample analysis, the spiked distilled water was used in optimization of C_{18} SPE. The results of optimization indicated that addition of organic solvent into the spiked water sample could improve the percent recoveries of all studied PCBs. Increasing amount of added organic solvent could increase the percent recoveries of almost all of studied PCBs. Decreasing the sample volume was found to provide better recoveries in some studied PCBs. Therefore the optimal conditions were 20 ml of water mixed with 10 ml of the solvent mixtures, ethylacetate : acetone : methanol

(1 : 2 : 2 v/v). The solution was loaded onto 500 mg of C_{18} SPE which had been conditioned with dichloromethane, methanol and solvent mixtures of water : ethyl acetate : acetone : methanol (10 : 1 : 2 : 2 v/v). Isooctane was used as the eluent. The quantification was performed on GC/ECD with a DB-1701 capillary column. The temperature program was 100°C - 100°C (1 min) - 190°C (10°C min^{-1}) - 250°C (5°C min^{-1}) - 250°C (10 min). The detection limits of all studied PCBs were in the range of 0.06-0.18 μg l^{-1}. The optimum conditions were used in the analysis of spiked and non-spiked drinking and natural surface water. No PCBs were detected in all of the water samples analyzed.