CHAPTER IV

RESULTS AND DISCUSSION

4.1 The Study of Tetrabutylammonium Bromide-2-Mercaptoethanol System

The preliminary study of various effects on the resolution of mercury compounds is performed in the mobile phase compose of methanol-water 30:70% v/v. With this composition all peaks of mercury compounds are detected and the resolution between inorganic and methylmercury is high enough for the observing slightly change from the study. The various effects in the preliminary study include mobile phase pH, tetrabutylammonium bromide (TBABr) concentration, and 2-mercaptoethanol concentration. These parameters are studied in order to observe the change of peak retention time, selectivity factor, capacity factor, peak resolution, and peak shape of mercury compounds.

The mobile phase pH is the first parameter for the study. The mobile phase pH in the range 2.0-8.0 is studied to find the suitable pH range for the next study. The mixture of methanol-water (30:70% v/v) containing 0.0050% v/v of 2-mercaptoethanol and 0.0050M of tetrabutylammonium bromide (TBABr) is initially served as mobile phase for the study. Each mobile phase is adjusted with hydrochloric acid and sodium hydroxide solution to the desirous pH. The results of the effect of mobile phase pH and the chromatograms are presented in Table A-2 and Figure A-1 in appendix section. From the study, the good resolutions between inorganic and methylmercury are found in pH range 2.0-4.0, but the pH above 4.0 the resolutions are decreased. The pH 2.0 gives the highest peak resolution, however tailing peak of methylmercury is found. The optimum pH range is found from the pH that gives high resolution and symmetrical peak shapes. It is found that the optimum pH range from the study is 3.0-4.0. The pH value of mobile phase adjusted with hydrochloric acid and sodium hydroxide solution is unstable, so the acetate buffer is used to stabilise and exactly control the pH value. The mobile phase pH in the range 3.00-5.00 is studied in order to find the optimum mobile phase pH. The results of the effect of mobile phase pH and the chromatograms are presented in Table A-4 and Figure A-2. From the study, no significant differences in peak resolution and peak shape are obtained in pH 3.00-5.00. However, the mobile phase with pH 3.00 gives the highest peak resolution between inorganic mercury and methylmercury. Therefore, pH 3.00 is chosen for the next study.

The second parameter in the study is the 2-mercaptoethanol concentration. The 2-mercaptoethanol concentration is studied under the mobile phase composed of methanol-water 30:70% v/v buffered with 1.00 x 10^{-3}M acetate-acetic acid pH 3.00 containing 0.0050 M TBABr. The 2-mercaptoethanol concentrations are varied from 0.0010% v/v to 0.0075% v/v. The results and the chromatograms from this study are presented in Table A-6 and Figure A-3. From the study, in low concentration of 2-mercaptoethanol (below 0.0040% v/v), the incomplete resolution between inorganic and methylmercury is observed and tailing peaks are found. The increased resolution and symmetric peak are obtained at concentration 0.0040% v/v. Inorganic and methylmercury are successfully separated in 0.0050% v/v of 2-mercaptoethanol. However, higher concentrations (above 0.0060% v/v) give higher noise of baseline signal. Therefore, the optimum 0.0050% v/v of 2-mercaptoethanol concentration is chosen for the next study.

The effects of tetrabutylammonium bromide concentration are the next study. The mixtures of methanol-water (30:70% v/v) buffered with 1.00 x 10^{-3} M acetate-acetic acid buffer pH 3.00 containing 0.0050% v/v 2-mercaptoethanol and TBABr concentration varied from 0 M to 0.0150M are served as the mobile phase in this study. The results and the chromatograms are presented in Table A-8 and Figure A-4. From the study in the absence of TBABr, the resolution between inorganic and methylmercury is higher than the addition of TBABr in mobile phase. On the other hand, asymmetric peaks and unstable baseline signal are found in this mobile phase system. In the low TBABr concentration (below 0.0050M), tailing peaks of inorganic mercury and methylmercury are found. The incomplete resolution is also found in this concentration. When the concentration is increased, the higher resolution and more symmetric peak are found. But at high concentration (above 0.0100M) lower resolution and higher noise signal are observed. Therefore, the optimum 0.0075M tetrabutylammonium bromide concentration is chosen for the next study.

The studies above show that peaks of inorganic and methylmercury are effected from the change in pH, 2-mercaptoethanol concentration, and TBABr concentration. On the other hand, the effects of peak of phenylmercury are not significantly observed. Because the slightly change in percent methanol in mobile phase, the retention time and peak shape of phenylmercury are greatly changed. The mobile phase compose of methanol-water (30:70% v/v) is suitable for the separation inorganic and methylmercury, but unsuitable for phenylmercury. While percent methanol is increased, inorganic and methylmercury are not successfully separated. This mobile phase system, tetrabutylammonium bromide-2-mercaptoethanol system, is unsuitable for the determination of phenylmercury. Consequently, this mobile phase system is studied in order to optimise for the separation only inorganic and methylmercury.

4.1.1 The Study of Various Effects on the Resolution of Mercury Compounds

Various effects on the resolution of inorganic and methylmercury, i.e. methanol composition in mobile phase, mobile phase flow rate the mobile phase pH, 2-mercaptoethanol concentration, and tetrabutylammonium bromide concentration are studied. The optimum chromatographic conditions and the observation the effects on the peak shapes of inorganic and methylmercury are the objectives of the study.

4.1.1.1The Effect of Methanol Composition in Mobile Phase

The effects of the methanol composition in mobile phase are studied in order to find the optimum mobile phase conditions for the separation inorganic and methylmercury. The retention mechanism of inorganic and methylmercury is also studied. The mixtures of methanol-water buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 3.00 containing 0.0075M TBABr and 0.0050% v/v 2-mercaptoethanol are served as the mobile phase composition. In this study the proportion of methanol-water are varied from 10:90% v/v to 70:30% v/v. The chromatographic conditions are shown in Table 3.1.1 according to experimental section 3.4.1.1. The results of this study are presented in Table 4.1.1 and Figures 4.1.1-4.1.3. Form Table 4.1.1 and Figures 4.1.1-4.1.3, the retention time and peak resolution of mercury compounds are changed with methanol percentage. The trend of retention time and peak resolution are decreased, when the proportion of methanol is increased. At the low proportion (10-20% v/v) of methanol the high retention time of mercury compounds is found. Phenylmercury is not detected in this system because of the retention time more than 30 minutes and peak broadness. When the proportion of methanol is increased, the retention time and peak resolution of mercury compounds are decreased. The sharper peak shape of phenylmercury is detected, but inorganic and methylmercury are not separated in the high proportion of methanol (above 70% v/v). Inorganic and methylmercuy are separated, when the proportion of methanol is decreased. However, the elution order of inorganic and methylmercuryl is changed. Methylmercury is eluted before inorganic mercury in low proportion of methanol (below 20% v/v). The good resolutions of inorganic and methylmercury are obtained in 10%-15% methanol. The highest resolution of mercury compounds is found in 10% of methanol in mobile phase, but the high retention times are also observed. The peak of inorganic and methylmercury is completely overlap in 20% v/v of methanol. It is found that the optimum retention time, peak resolution, and peak shape are obtained in the mobile phase composed of methanol-water 12:88% v/v. Therefore, 12% methanol in mobile phase is chosen for the next study.

Table 4.1.1: The results of the effect of the methanol composition in mobile phase on the chromatographic parameters for the separation of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (Triplicate analyses)

%MeOH	compound	t_R (min)	k′	α*	R_s**
10	solvent	2.984±0.004	-	-	
	MeHg$^+$	10.563±0.051	2.540	1.229	4.186
	Hg^{2+}	12.296±0.137	3.121		
12	solvent	2.932±0.114	-	-	
	MeHg$^+$	9.790±0.091	2.339	1.173	2.614
	Hg^{2+}	10.974±0.191	2.743		
15	solvent	2.924±0.079	-	-	
	MeHg$^+$	9.261±0.015	1.980	1.904	1.054
	Hg^{2+}	8.714±0.020	2.167		
20	solvent	2.838±0.005	-	-	
	Hg^{2+}	7.309±0.007	1.575	1.000	0.000
	MeHg$^+$				
30	solvent	2.904±0.005	-		
	Hg^{2+}	5.143±0.060	0.771	1.191	1.163
	MeHg$^+$	5.571±0.048	0.918		
40	solvent	2.876±0.004	-		
	Hg^{2+}	4.280±0.016	0.488	1.189	1.180
	MeHg$^+$	4.719±0.025	0.641		
50	solvent	2.863±0.006	-		
	Hg^{2+}	3.820±0.059	0.334	1.224	0.873
	MeHg$^+$	4.033±0.021	0.409		
60	solvent	2.877±0.042	-		
	Hg^{2+}	3.380±0.017	0.175	1.416	0.905
	MeHg$^+$	3.647±0.007	0.268		
70	solvent	2.858±0.003	-	-	
	Hg^{2+}	3.401±0.011	0.190	1.00	0
	MeHg$^+$				

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

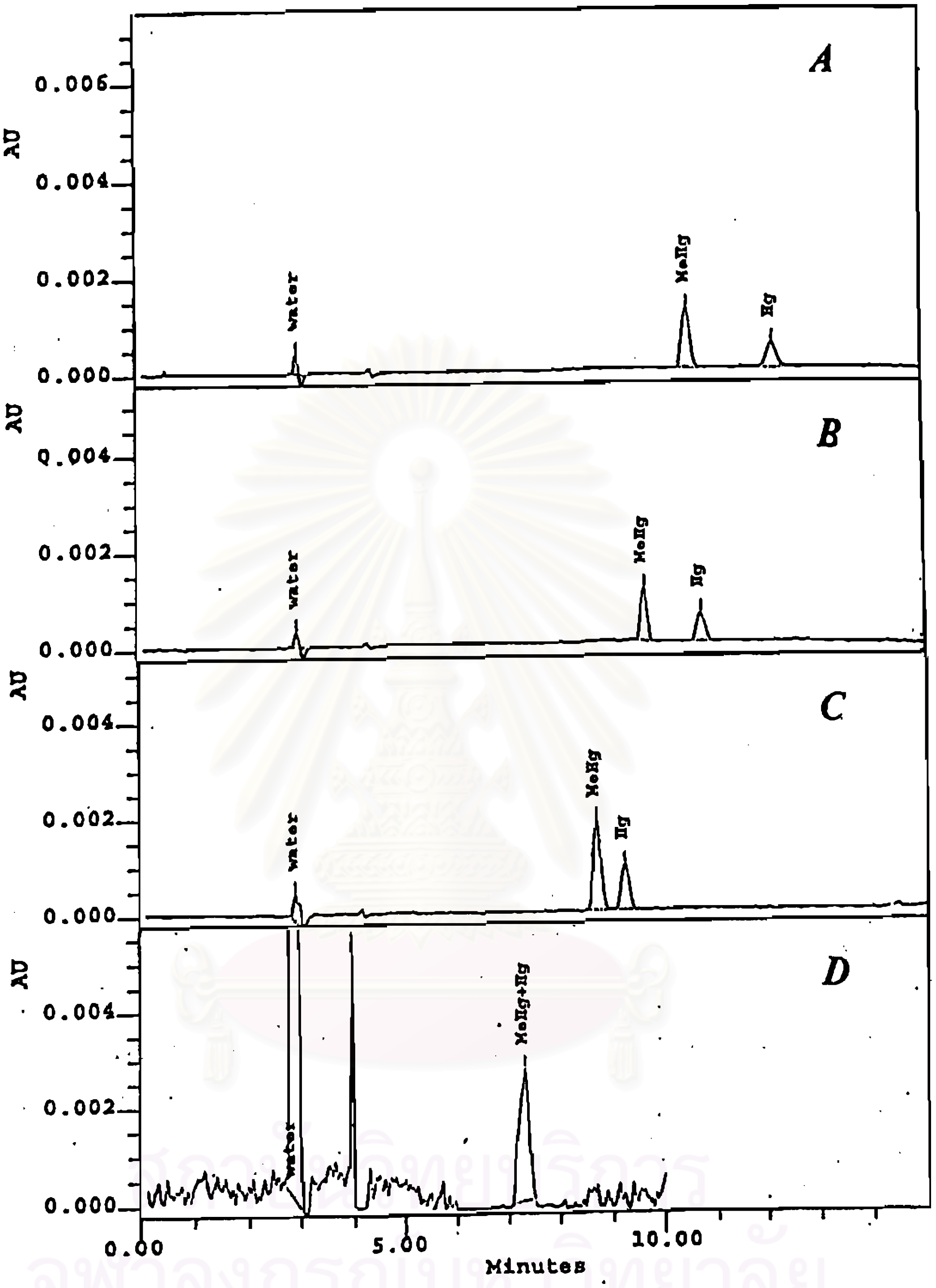


Figure 4.1.1: The chromatograms of inorganic and methylmercury at variety of methanol composition in mobile phase in tetrabutylammonium bromide-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.1.1)

A) 10% B) 12% C) 15% D) 20%
E) 40% F) 50% G) 60% H) 70%

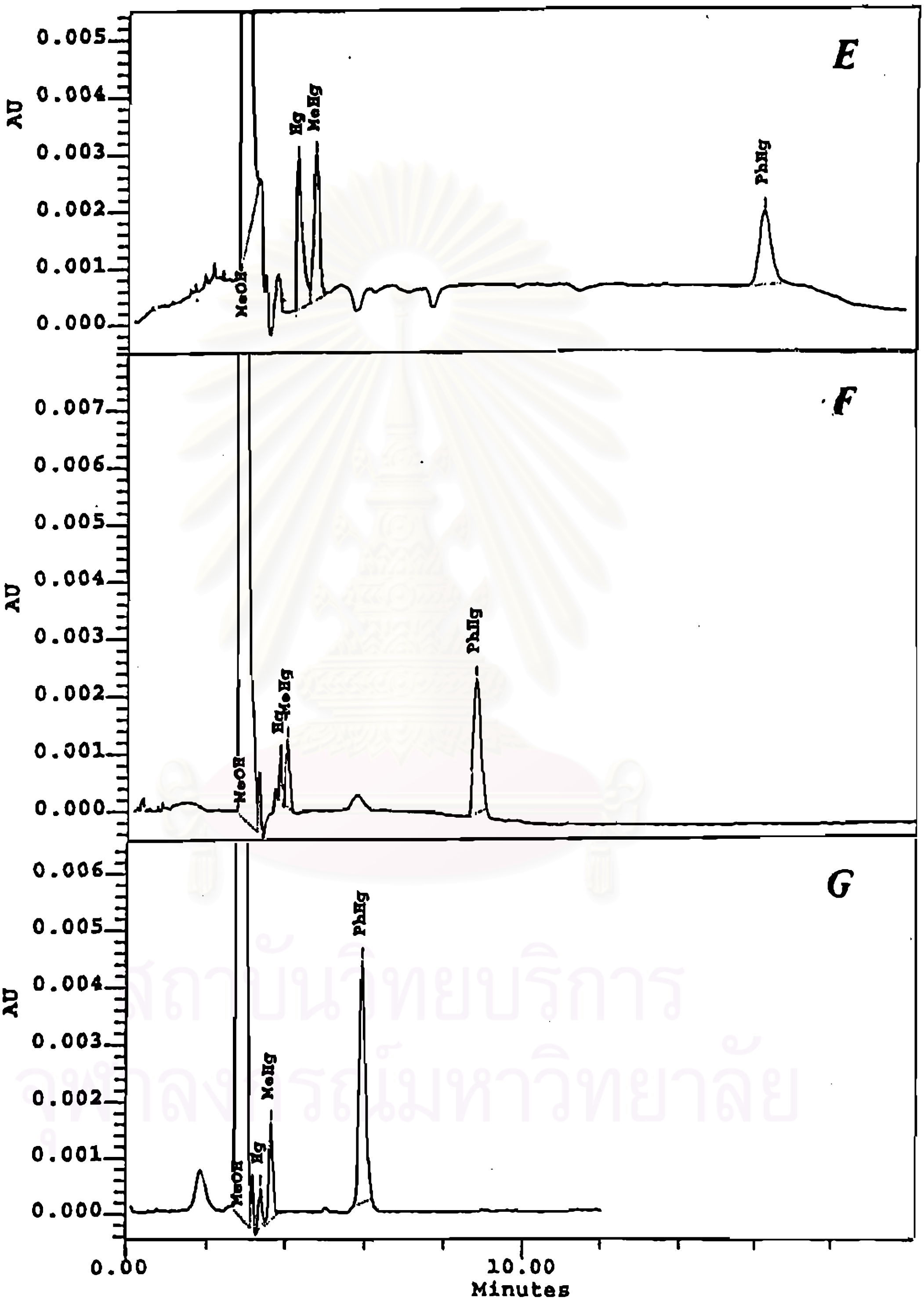
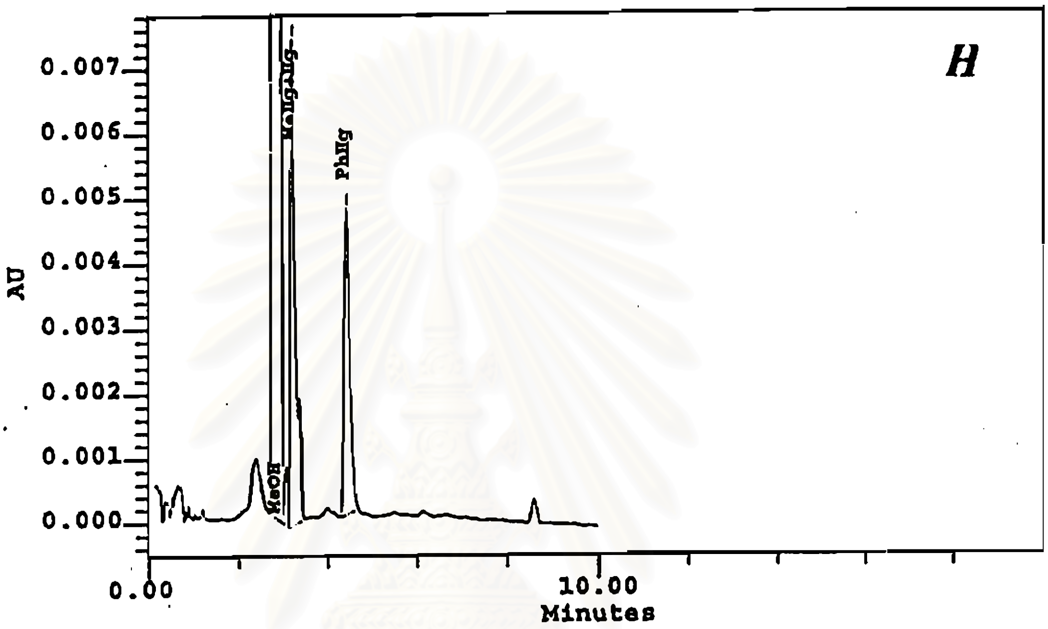


Figure 4.1.1 (continue)



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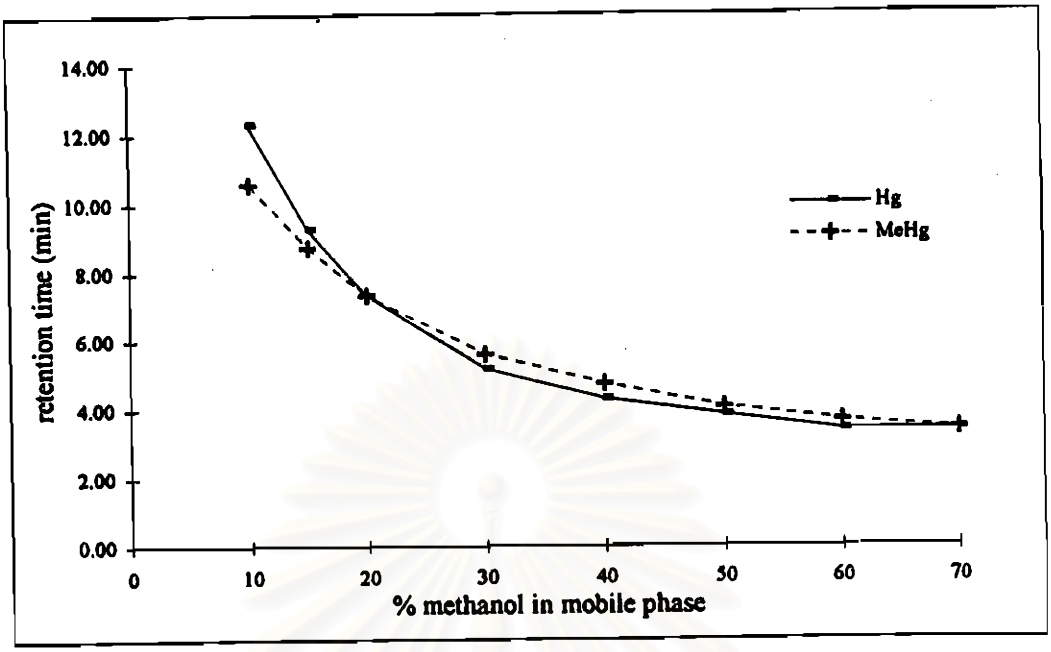

Figure 4.1.2: The relationship between the retention time of inorganic and methylmercury and percent methanol in mobile phase.

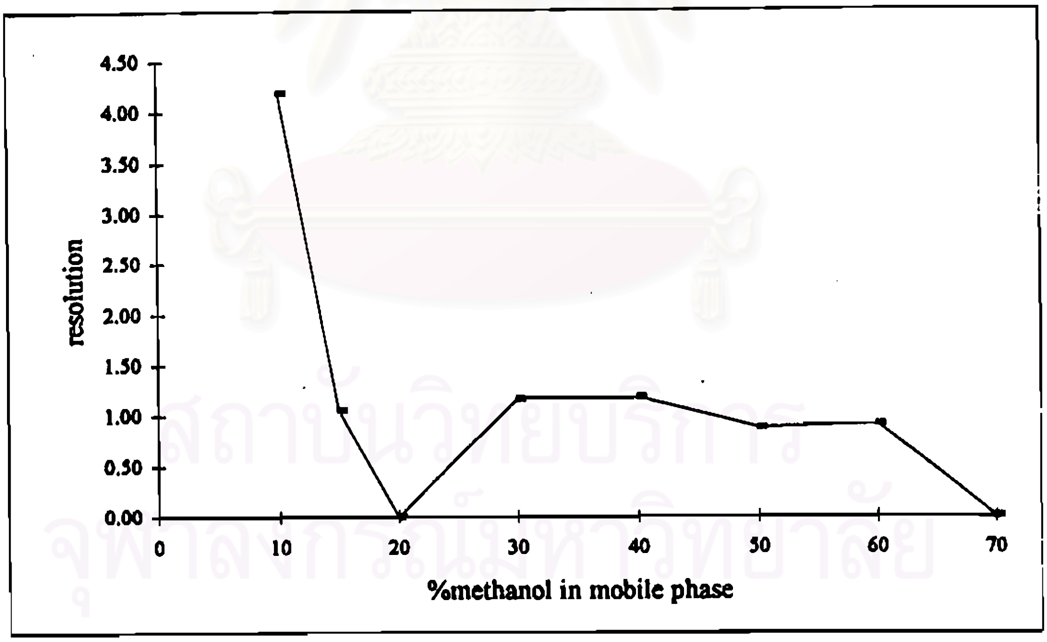

Figure 4.1.3 The relationship between the resolution of inorganic and methylmercury and percent methanol in mobile phase

The effects of the methanol composition in mobile phase of tetrabutylammomium bromide-2-mercaptoethanol system are compared with the 2-mercaptoethanol system. The mixture of methanol-water buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 3.00 containing 0.0050% v/v 2-mercaptoethanol are served as the mobile phase of 2-mercaptoethanol system. The proportion of methanol-water are varied from 10:90% v/v to 70:30% v/v as in the tetrabutylammonium bromide-2-mercaptoethanol system. The results of 2-mercaptoethanol system are shown in Table 4.1.2. The comparisons are show in Figures 4.1.4-4.1.6.

Table 4.1.2: The effect of methanol composition in mobile phase on the chromatographic parameters for the separation to inorganic and methylmercury compounds in 2-mercaptoethanol system. (Triplicate analyses)

%MeOH	compound	t_R (min)	k'	α*	R_S**
10	solvent	3.056±0.026	-	0.922	1.001
	$MeHg^+$	10.976±0.026	2.592		
	Hg^{2+}	11.644±0.072	2.810		
15	solvent	3.003±0.050	-	1.000	0
	Hg^{2+}	8.662±0.035	1.884		
	$MeHg^+$				
20	solvent	2.949±0.009	-	1.132	1.319
	Hg^{2+}	6.644±0.110	1.253		
	$MeHg^+$	7.132±0.077	1.418		
30	solvent	2.904±0.008	-	1.288	1.398
	Hg^{2+}	4.934±0.056	0.699		
	$MeHg^+$	5.517±0.039	0.900		
40	solvent	2.875±0.003	-	1.600	1.535
	Hg^{2+}	3.932±0.011	0.368		
	$MeHg^+$	4.569±0.008	0.589		
50	solvent	2.858±0.006	-	1.760	1.392
	Hg^{2+}	3.500±0.008	0.225		
	$MeHg^+$	3.990±0.005	0.396		
60	solvent	2.835±0.005	-	1.299	0.806
	Hg^{2+}	3.668±0.005	0.294		
	$MeHg^+$	3.918±0.051	0.382		
70	solvent	2.822±0.002	-	1.000	0
	Hg^{2+}	3.437±0.031	0.219		
	$MeHg^+$				

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

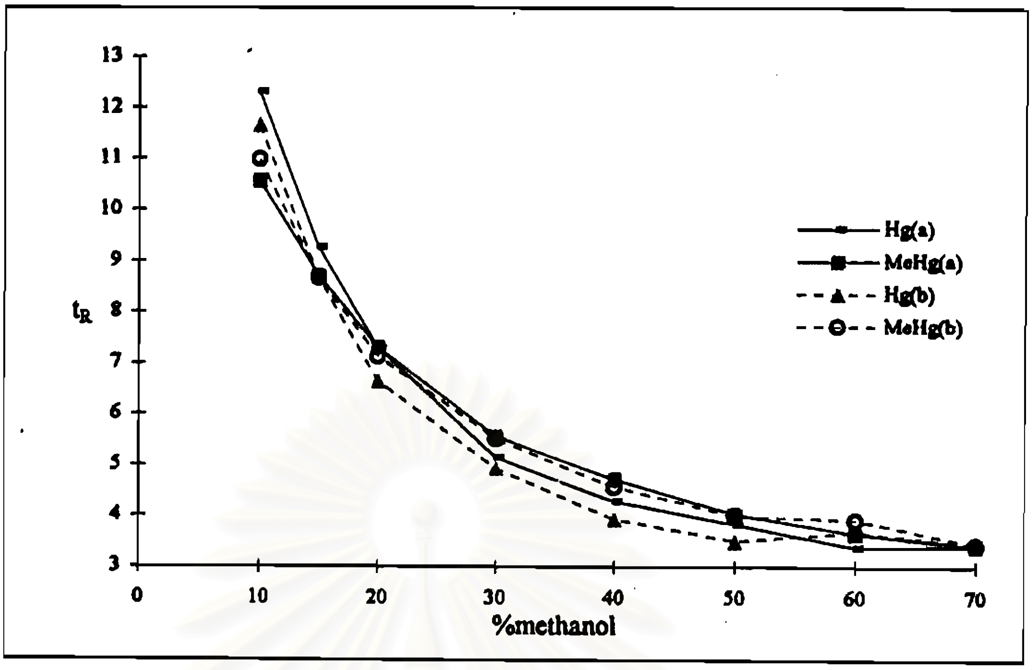

Figure 4.1.4: The relationship between retention time of inorganic and methylmercury with percent methanol in (a) tetrabutylammonium bromide-2-mercaptoethanol system and (b) 2-mercaptoethanol

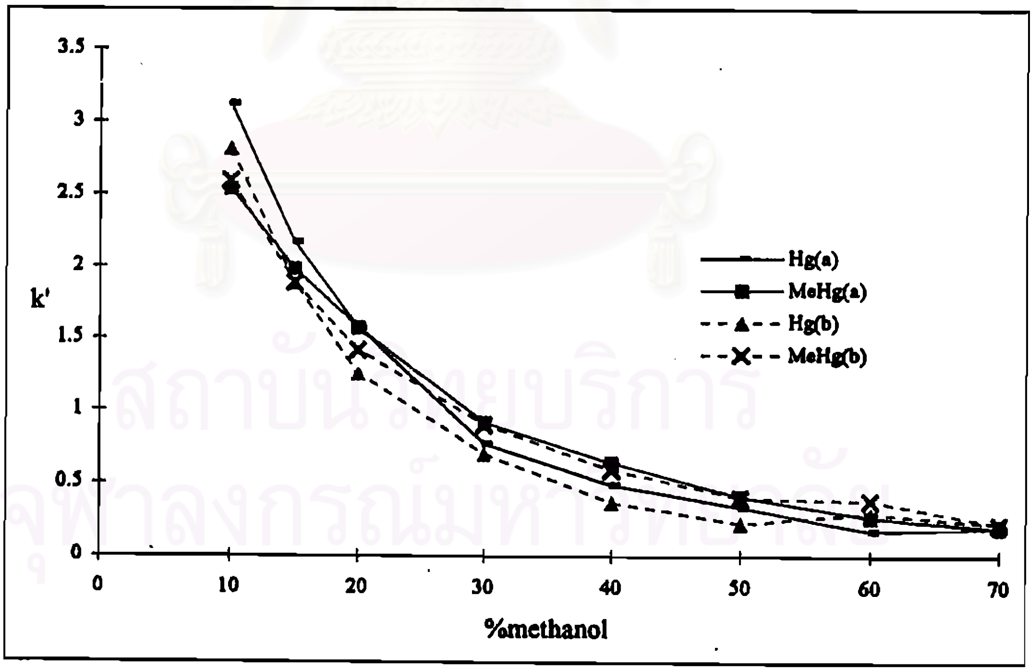

Figure 4.1.5: The relationship between capacity factor of inorganic and methylmercury with percent methanol in (a) tetrabutyl ammonium bromide-2-mercaptoethanol system and (b) 2-mercaptoethanol system

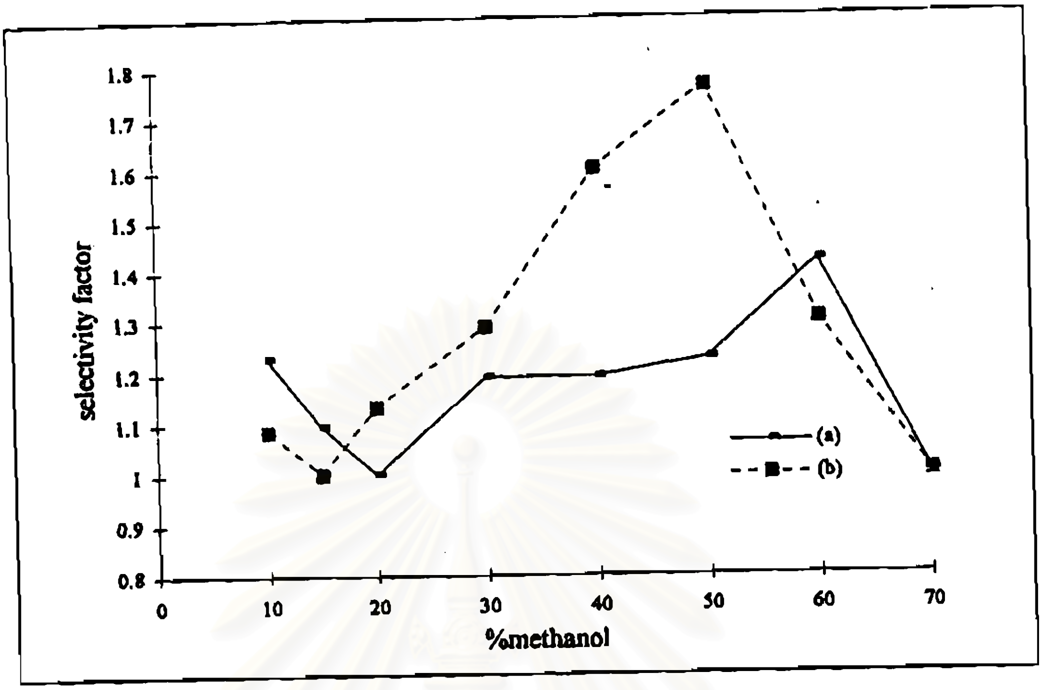

Figure 4.1.6: The relationship between selectivity factor of inorganic and methylmercury with percent methanol in (a) tetrabutyl ammonium bromide-2-mercaptoethanol system and (b) 2-mercaptoethanol system

In the mobile phase containing complexing agent and ion-pairing agent, both complexation retention mechanism and ion-pair retention mechanism are found. In mobile phase containing ion-pairing agent, TBABr, the Br^- forms negatively charge complexes with the mercury compounds, $HgBr_4^{2-}$ and $MeHgBr_2^-$, and then these complexes are separated by ion-pair formation with tetrabutylammonium ion. In the case of 2-mercaptoethanol, mercury-mercaptoethanol complexes are formed, and then are separated in the C_{18} stationary phase. In tetrabutylammonium bromide-2-mercaptoethanol system, the competition between the formation of bromide-complexes and mercaptoethanol-complexes are occurred. Considering the stability constant for both formations. The stability constants for bromide-complex are 10^6-10^8, whereas for mercaptoethanol-complexes are 10^{16}. It is show that the mercaptoethanol-complexes are stronger than the bromide-complexes. Thus, in this mobile phase system the mercaptoethanol-complexes are dominatingly formed. The retention mechanisms of mercury compounds are combination of both mechanisms. The relative contributions of these mechanisms depend on the mobile phase composition. The eluting strength and concentration of the methanol are the most important parameters in controlling elution and selectivity of mercury compounds. Considering tetrabutylammonium ion in mobile phase, the tetrabutylammonium ion can be partitioned into the C_{18} surface, it is the dynamic-coating in non-polar stationary phase. Two properties are shown in the modified stationary phase, the dynamic-anion exchange property from ammonium ion and the non-polar property from butyl-chain. When the low proportion of methanol, the hydroxyl groups of mercury-mercaptoethanol complexes can be ionised. In this case, the complexes is induced to the more polar molecules. Therefore, the retention is effected from the ionised group of mercaptoethanol ligand. Inorganic mercury forms complex with two ligands molecules, but methylmercury forms complex with one ligand. The comparison of inorganic with methylmercury shows that the inorganic complex has more ionisable groups (2 hydroxyl group) than methylmercury (1 hydroxyl group). Thus, inorganic mercury is stronger retained than methylmercury. An increase in methanol concentration, the ionic property of tetrabutylammonium ion is stabilised by methanol molecules. In this case, the complexes have the dominated-neutral property. Therefore, the retention is effected from the non-polar size of the complexes molecule. The methylmercury-complex has the methylene group, so it is the non-polar complex than inorganic mercury. Thus, methylmercury is stronger retained in the non-polar stationary phase and has longer retention time. When compare the tetrabutylammonium bromide-2-mercaptoethanol system with the 2-mercaptoethanol system, the retention time of mercury compounds in the former system are longer than the later system. This results from the butyl-chain of tetrabutylammonium bromide increases the non-polar property of C_{18} surface to C_{22} surface, thus the partitions of mercury complexes molecule are stronger than the conventional C_{18}.

4.1.1.2 The Effect of Mobile Phase Flow Rate

The previous study, inorganic and methylmercury are eluted in the long retention time. The mobile phase flow rates are studied to find the optimum flow rate that gives the reasonable separation time. The mixture of methanol-water (12:88% v/v) buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer solution pH 3.00 containing 0.0050% v/v 2-mercaptoethanol and 0.0075M TBABr is served as the mobile phase according to experimental section 3.4.2 and Table 3.1.2. The effects of mobile phase flow rate on the retention time, peak resolution, and peak shape of mercury compounds are presented in Table 4.1.3 and Figure 4.1.7. From the results show that the flow rates from 1.00 to 1.80 mL/min are essentially no effected on the capacity factor and selectivity factor. However, the retention time and peak resolution are affected. From the previous studies, the complete resolution of mercury compounds is obtained in the low proportion of methanol, but the long retention times of inorganic and methylmercury and the peak broadness are observed. The decreased retention time and sharper peak shape are obtained, when the flow rate is increased. All of the study flow rates give the good resolution, but the pressure of HPLC system is rapidly increased with the high flow rate. It is found that the optimum retention time, peak resolution, peak shape, and pressure of HPLC system is obtained in flow rate 1.50 mL/min. Therefore, the flow rate of 1.50 mL/min is chosen for the next study.

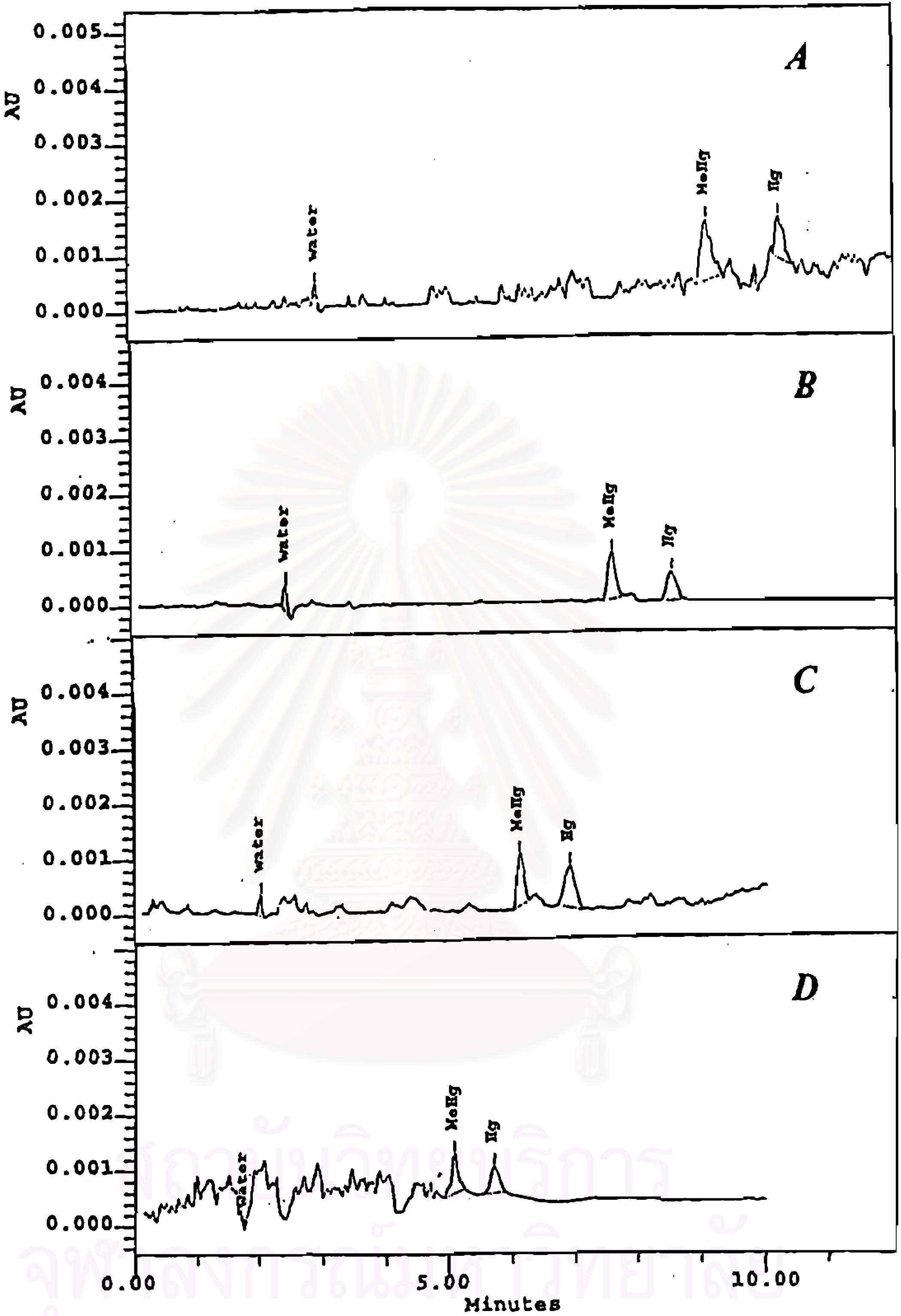

Figure 4.1.7: The chromatograms of inorganic and methylmercury variety of mobile phase flow rate in tetrabutylammonium bromide-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.1.6)

A) 1.00 mL/min B) 1.20 mL/min

C) 1.50 mL/min D) 1.80 mL/min

Table 4.1.3: The results of the effect of mobile phase flow rate on the chromatographic parameters for the separation of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (replicate analyses, n=5)

flow rate (mL/min)	compound	t_R (min)	W (min)	k′	α*	R_s**
1.00	solvent	2.928±0.032	-	-	1.128	2.834
	$MeHg^+$	9.092±0.010	0.367±0.068	3.105		
	Hg^{2+}	10.260±50.048	0.457±0.083	3.504		
1.20	solvent	2.471±0.001	-	-	1.123	2.269
	$MeHg^+$	7.591±0.007	0.347±0.062	3.072		
	Hg^{2+}	8.528±0.009	0.480±0.136	3.451		
1.50	solvent	1.946±0.100	-	-	1.131	2.286
	$MeHg^+$	6.098±0.038	0.314±0.086	3.134		
	Hg^{2+}	6.898±0.097	0.386±0.070	3.545		
1.80	solvent	1.675±0.104	-	-	1.125	2.064
	$MeHg^+$	5.119±0.026	0.357±0.076	3.056		
	Hg^{2+}	5.759±0.050	0.264±0.068	3.438		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

4.1.1.3 The Effect of Mobile Phase pH

From the previous study, the mixture of methanol-water (12:88% v/v) buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 3.00 at the optimum flow rate 1.50 mL/mim. The effects of mobile phase pH on the resolution and peak shapes of inorganic and methylmercury are studied according to experimental section 3.4.1.3 and Table 3.1.3. The acetate-acetic acid buffer solution is chosen in order to control the pH of the mobile phase. Because the mobile phase pH adjusted with hydrochloric acid and sodium hydroxide solution is unstable. The pH 3.00-5.00 are studied to find the optimum pH that the mixed complexing agent and ion-pairing agent can be form complex or ion pair appositely with inorganic and methylmercury. The effects of mobile phase pH on the resolution of mercury compounds are presented in Table 4.1.4 and Figure 4.1.8. At pH 5.00 the highest peak resolution is obtained; however, the tailing peak of methylmercury is observed. In pH 3.00-4.00, no significant difference in peak resolution is observed. The pH 3.00 gives the symmetrical peak shape both inorganic and methylmercury. While pH 3.50 the tailing peak of inorganic mercury and pH 4.00 the slightly tailing peak of methylmercury are found. It is found that the optimum peak resolution and peak shape are obtained in pH 3.00 of 1.00 x 10^{-3}M acetate-acetic acid buffer.

Table 4.1.4: The results of the effect of mobile phase pH on the chromatographic parameters for the separation of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (Triplicate analyses)

pH	compounds	t_R (min)	W (min)	k'	α*	R_S**
3.00	solvent	2.043±0.025	-	-	1.198	2.116
	$MeHg^+$	6.727±0.021	0.445±0.101	2.293		
	Hg^{2+}	7.656±0.043	0.433±0.118	2.747		
3.50	solvent	2.031±0.010	-	-	1.213	2.234
	$MeHg^+$	6.774±0.061	0.439±0.048	2.335		
	Hg^{2+}	7.784±0.123	0.406±0.094	2.832		
4.00	solvent	2.027±0.007	-	-	1.203	2.153
	$MeHg^+$	6.737±0.074	0.441±0.067	2.323		
	Hg^{2+}	7.691±0.164	0.446±0.086	2.794		
5.00	solvent	2.022±0.021	-	-	1.260	2.916
	$MeHg^+$	6.693±0.043	0.383±0.044	2.310		
	Hg^{2+}	7.909±0.163	0.451±0.029	2.911		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

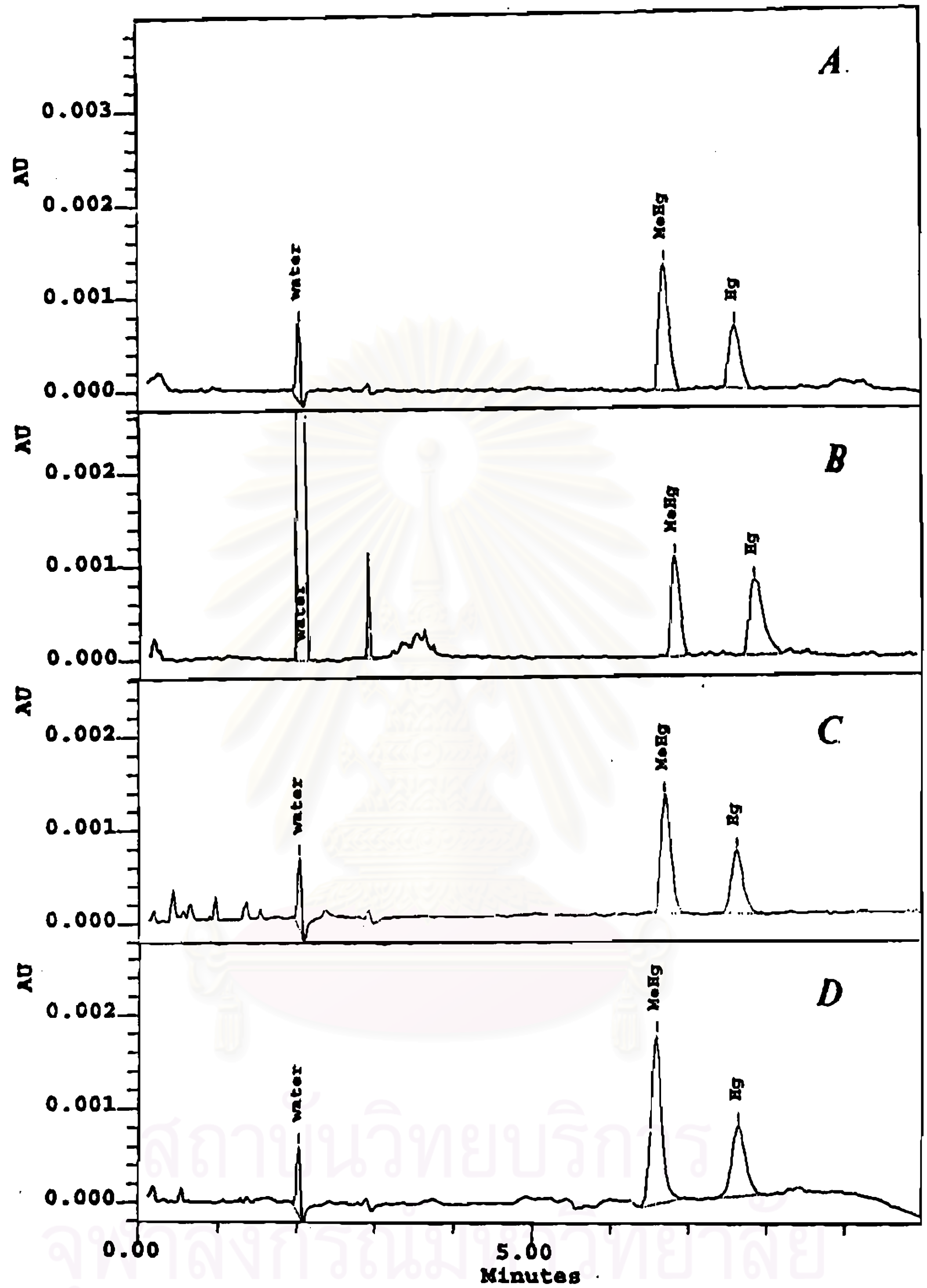

Figure 4.1.8: The chromatograms of mercury compounds at variety of mobile phase pH with acetate-acetic acid buffered in tetrabutylammonium bromide-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.1.3)

A) pH 3.00 B) pH 3.50 C) pH 4.00 D) pH 5.00

4.1.1.4 The Effect of Acetate-Acetic Acid Buffer Concentration

The acetate-acetic acid buffer solution is used to control the mobile phase pH. The mixture of methanol-water (12:88% v/v) compose of 0.0050% v/v 2-mercaptoethanol and 0.0075M TBABr buffered with acetate-acetic acid buffer pH 3.00 is used as the mobile phase for the study. The buffer concentrations in this study are 1.00×10^{-3}, 2.00×10^{-3}, and 5.00×10^{-3} M according to experimental section 3.4.1.4 and Table 3.1.4. From the study found that the increased buffer concentrations are not affect to the peak resolution and peak shape, but the increased in baseline signal and long column equilibration time are observed. Therefore, the lowest buffer concentration from the study, 1.00×10^{-3} M, is chosen for the next study.

4.1.1.5 The Effect of 2-Mercaptoethanol Concentration

2-Mercaptoethanol is used as the complexing agent to form the strongly complexes with the mercury compounds in order to separate in C_{18} column. From the previous studies the optimum mobile phase pH is found as 3.00 with 1.00×10^{-3}M acetate-acetic acid buffer solution. The effects of 2-mercaptoethanol concentration in the mobile phase are studied in this section. The concentrations are varied in range 0.0040-0.0060% v/v according to experimental section 3.4.1.5. The mixture of methanol-water (12:88% v/v) buffered with 1.00×10^{-3}M acetate-acetic acid buffer pH 3.00 containing 0.0075M TBABr is fixed to use as the mobile phase system in this study. The chromatographic conditions of the study are shown in Table 3.1.5. The effects of 2-mercaptoethanol concentration on the resolution of mercury compounds are presented in Table 4.1.5 and Figure 4.1.9. The peak shapes of mercury compounds are effected from the 2-mercaptoethanol concentration. In concentration 0.0040% v/v the tailing peaks of inorganic and methylmercury are found. When the concentrations are increased, the symmetrical peaks are found. However, in higher concentration the noisy baseline and long column equilibration time are observed. It is indicate that the optimum peak resolution, peak shape, and the baseline signal are obtained in 0.0050% v/v of 2-mercaptoethanol. This concentration is chosen for the next study.

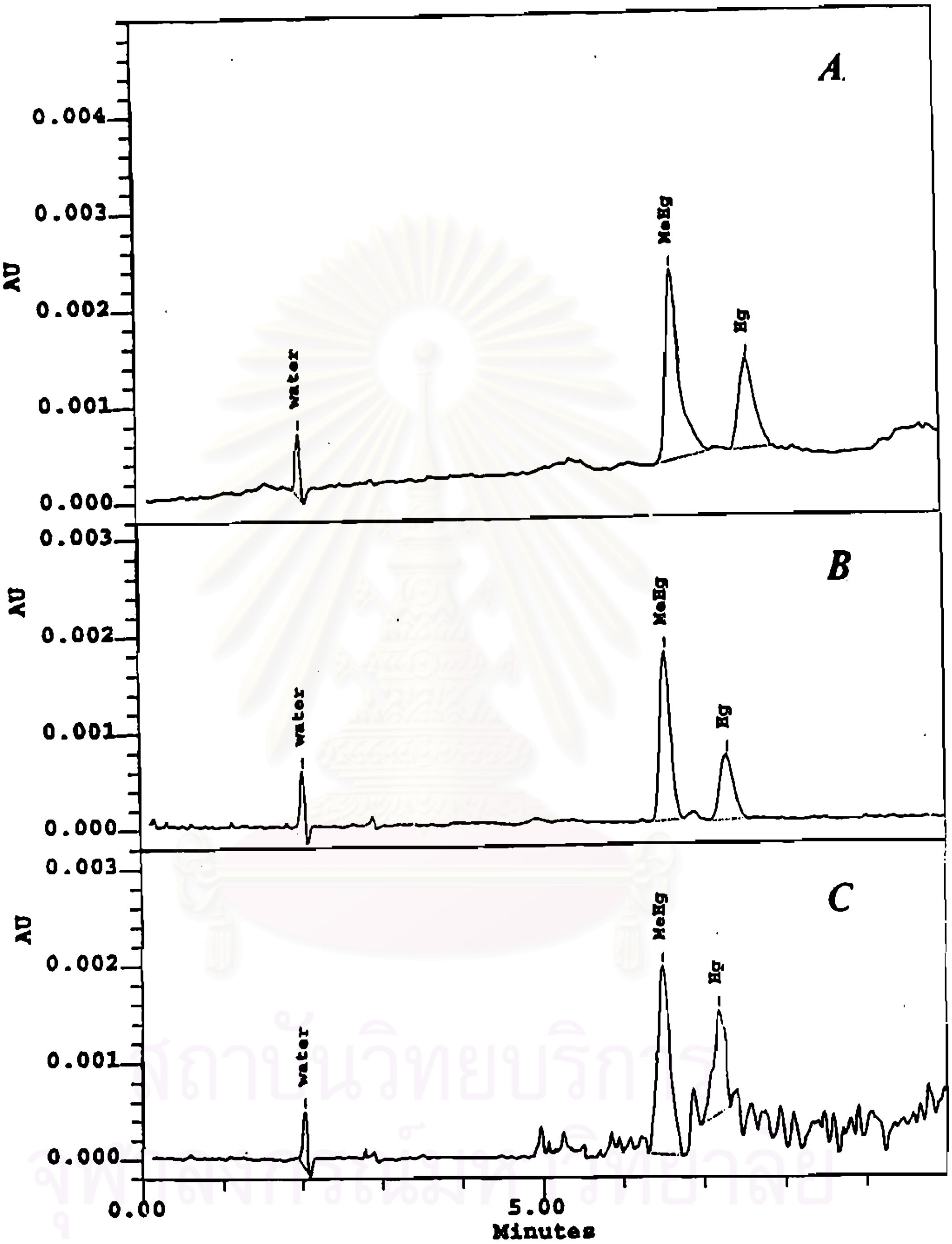

Figure 4.1.9: The chromatograms of inorganic and methylmercury at variety of 2-mercaptoethanol concentration in tetrabutylammonium bromide-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.1.5)

A) 0.0040% B) 0.0050% C) 0.0060%

Table 4.1.5: The results of the effect of 2-mercaptoethanol concentration on the chromatographic parameters for the separation of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (Triplicate analyses)

mercaptoethanol concentration	compounds	t_R (min)	W (min)	k'	α*	R_s**
0.0040%	solvent	2.027±0.005	-	-	1.195	1.695
	$MeHg^+$	6.690±0.010	0.557±0.118	2.300		
	Hg^{2+}	7.597±0.026	0.513±0.039	2.748		
0.0050%	solvent	2.029±0.006	-	-	1.165	1.690
	$MeHg^+$	6.552±0.006	0.413±0.043	2.229		
	Hg^{2+}	7.299±0.005	0.410±0.025	2.597		
0.0060%	solvent	2.024±0.004	-	-	1.157	1.638
	$MeHg^+$	6.521±0.026	0.450±0.070	2.222		
	Hg^{2+}	7.227±0.047	0.412±0.025	2.571		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

4.1.1.6 The Effect of Tetrabutylammonium Bromide Concentration

Tetrabutylammonium bromide (TBABr) is used as the ion-pairing agent in order to separate the ionic mercury compounds in C_{18} column. The effects of TBABr concentration in the mobile phase are studied. The mixtures of methanol-water (12:88% v/v) buffered with 1.00 x 10^{-3} M acetate-acetic acid buffer pH 3.00 containing 0.0050% v/v 2-mercaptoethanol and TBABr concentration varied from 0.0060M to 0.0100M are served as the mobile phase according to experimental section 3.4.1.6 and chromatographic conditions in Table 3.1.6. The effects of tetrabutylammonium bromide concentration on the resolution of mercury compounds are presented in Table 4.1.6 and Figure 4.1.10. From the study indicate that the retention time, capacity factor, selectivity factor of methylmercury, and peak resolution are effected from the tetrabutylammonium bromide concentration. In contrast, inorganic peak is not significantly effected. In lowest TBABr concentration, 0.0060M, the slightly peak tailing of methylmercury is found. At TBABr concentration 0.0100M the highest resolution is found, but tailing peak of methylmercury is found. The good peak resolution and peak shapes are obtained in the tetrabutylammonium bromide concentration 0.0075M and 0.0085M. However, the lower concentration, 0.0075M, is chosen for the next study.

Table 4.1.6: The results of the effect of tetrabutylammonium bromide concentration on the chromatographic parameters for the separation of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (Triplicate analyses)

TBABr concentration	compounds	t_R (min)	W (min)	k'	α*	R_s**
0.0060M	solvent	2.030±0.004	-	-	1.156	1.550
	$MeHg^+$	6.634±0.010	0.457±0.078	2.268		
	Hg^{2+}	7.350±0.022	0.467±0.097	2.621		
0.0075M	solvent	2.032±0.005	-	-	1.181	1.813
	$MeHg^+$	6.582±0.053	0.456±0.010	2.239		
	Hg^{2+}	7.407±0.042	0.454±0.123	2.645		
0.0085M	solvent	2.029±0.003	-	-	1.177	1.761
	$MeHg^+$	6.553±0.072	0.410±0.095	2.230		
	Hg^{2+}	7.356±0.163	0.503±0.145	2.625		
0.0100M	solvent	2.024±0.005	-	-	1.190	2.087
	$MeHg^+$	6.544±0.064	0.387±0.101	2.233		
	Hg^{2+}	7.404±0.142	0.436±0.086	2.658		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

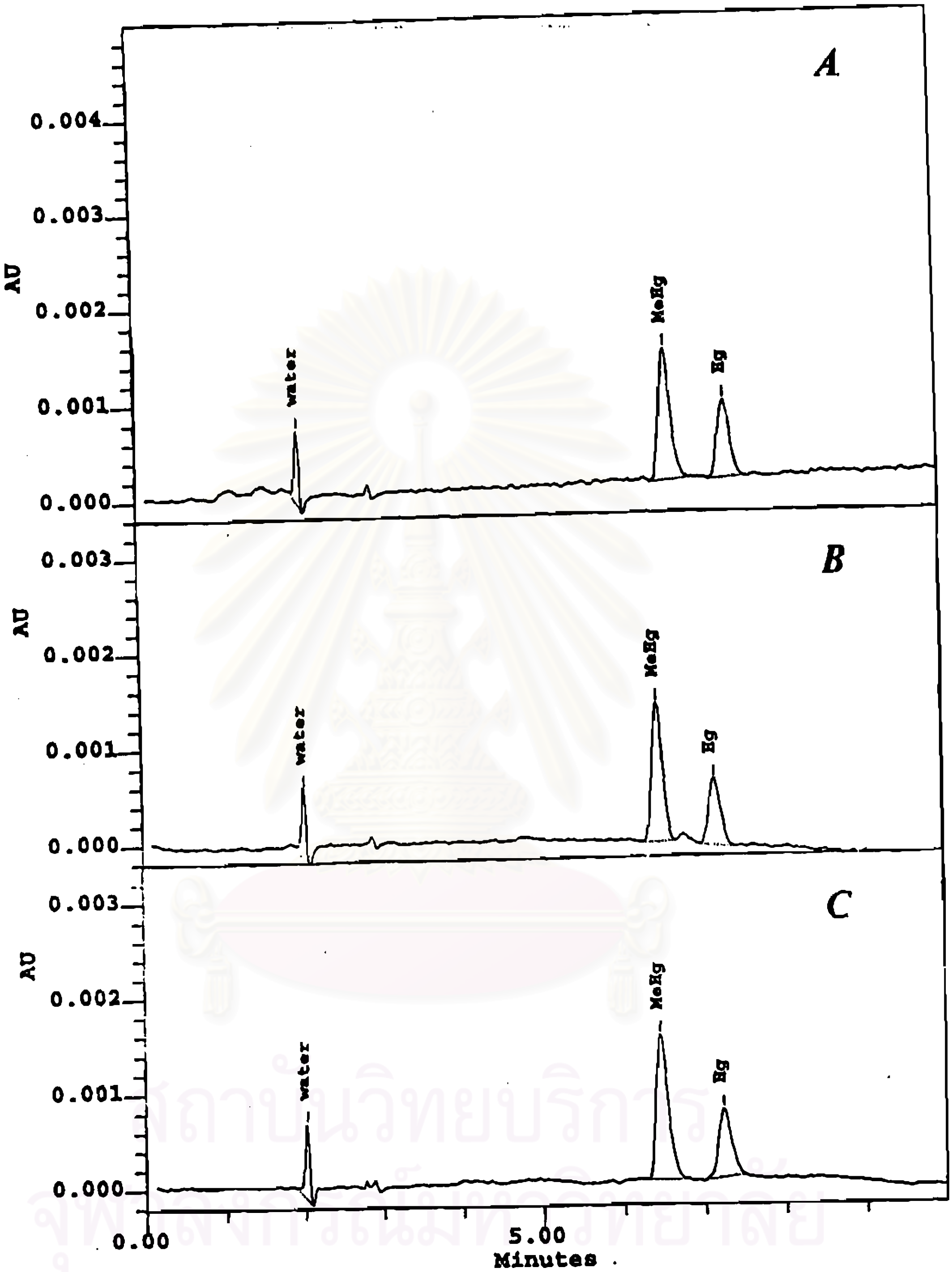

Figure 4.1.10: The chromatograms of inorganic and methylmercury at variety of tetrabutylammonium bromide concentration in tetrabutylammonium bromide-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.1.6)

A) 0.0060M B) 0.0075M C) 0.0100M

The injection volume is affected to peak height and peak area of mercury compounds. The effects of an injection volume at the optimum mobile phase composition are studied. The injection volume 10-100 µL are studied according to experimental section 3.4.2 and Table 3.1.7. The effects of an injection volume on the peak area and peak height response signals of mercury compounds are presented in Table 4.1.7 and Figures 4.1.11-4.1.13. It is found that the volume 50 µL give both the highest peak height signal and peak area signal per nanogram of mercury. The highest sensitivity of the mercury compounds is obtained in highest response signal. Therefore, the injection volume 50 µL is chosen as the optimum injection volume for the next study.

Table 4.1.7: The results of the effect of injection volume on the response signal of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (replicate analyses, n=5)

injection volume (µL)	compounds	Area (µV.sec)	response signal (µV.sec/ng)	Height (µV)	response signal (µV/ng)
10	Hg^{2+} $MeHg^+$	3698±1125 7967±2170	73.96 79.67	399±96 871±116	7.98 8.71
20	Hg^{2+} $MeHg^+$	7950±2678 14134±4042	79.50 70.67	879±256 1512±256	8.79 7.56
25	Hg^{2+} $MeHg^+$	13143±3034 20787±3191	105.14 83.15	1147±144 2269±212	9.18 9.08
50	Hg^{2+} $MeHg^+$	38185±7688 50046±6199	152.74 100.09	2975±281 4754±162	11.90 9.51
100	Hg^{2+} $MeHg^+$	73571±11407 89204±9468	147.14 89.20	5324±579 8669±510	10.65 8.67

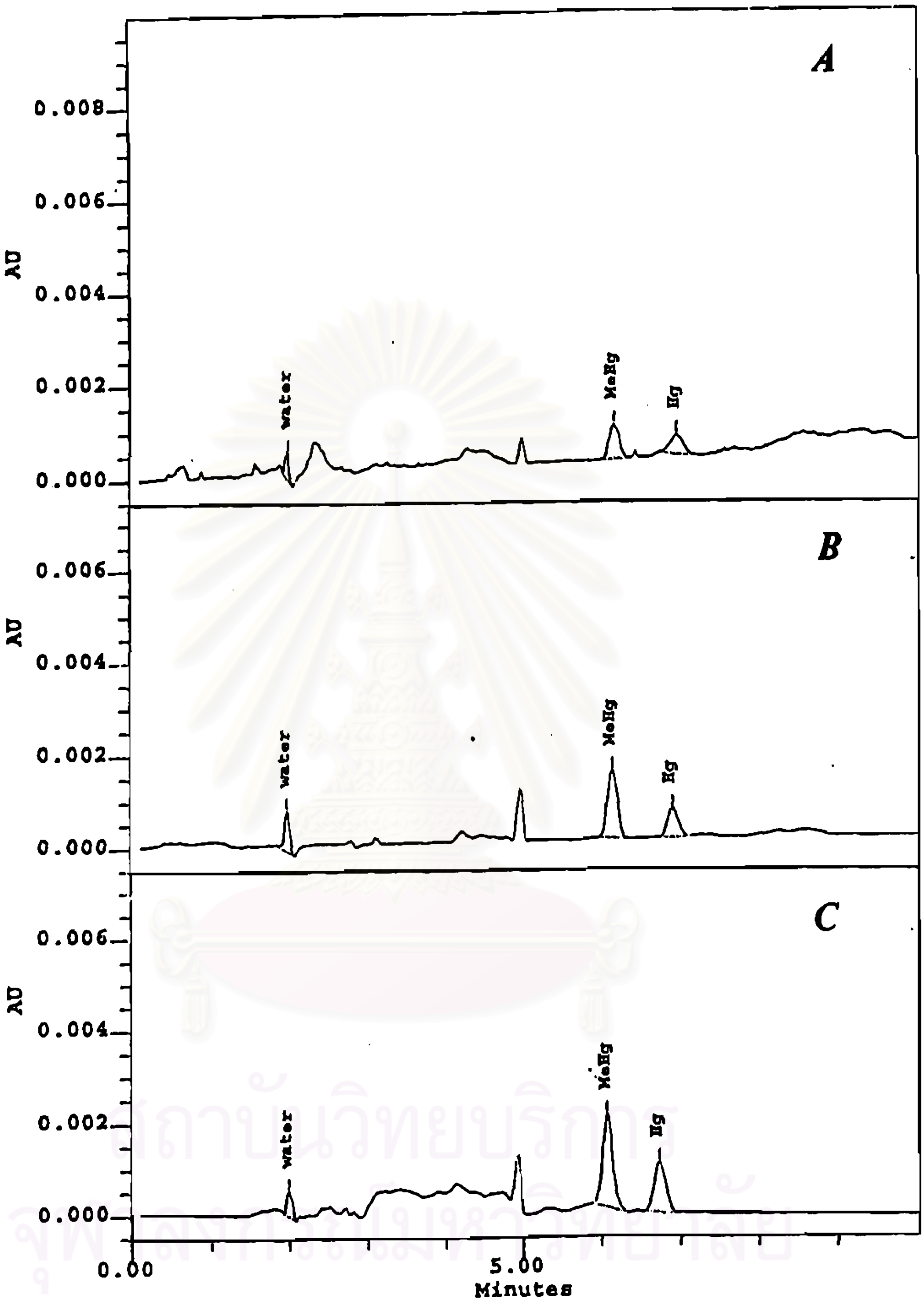

Figure 4.1.11: The chromatograms of inorganic and methylmercury at variety of injection volume in tetrabutylammonium bromide-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.1.7)

A) 10 μL B) 20 μL C) 25 μL D) 50 μL E) 100 μL

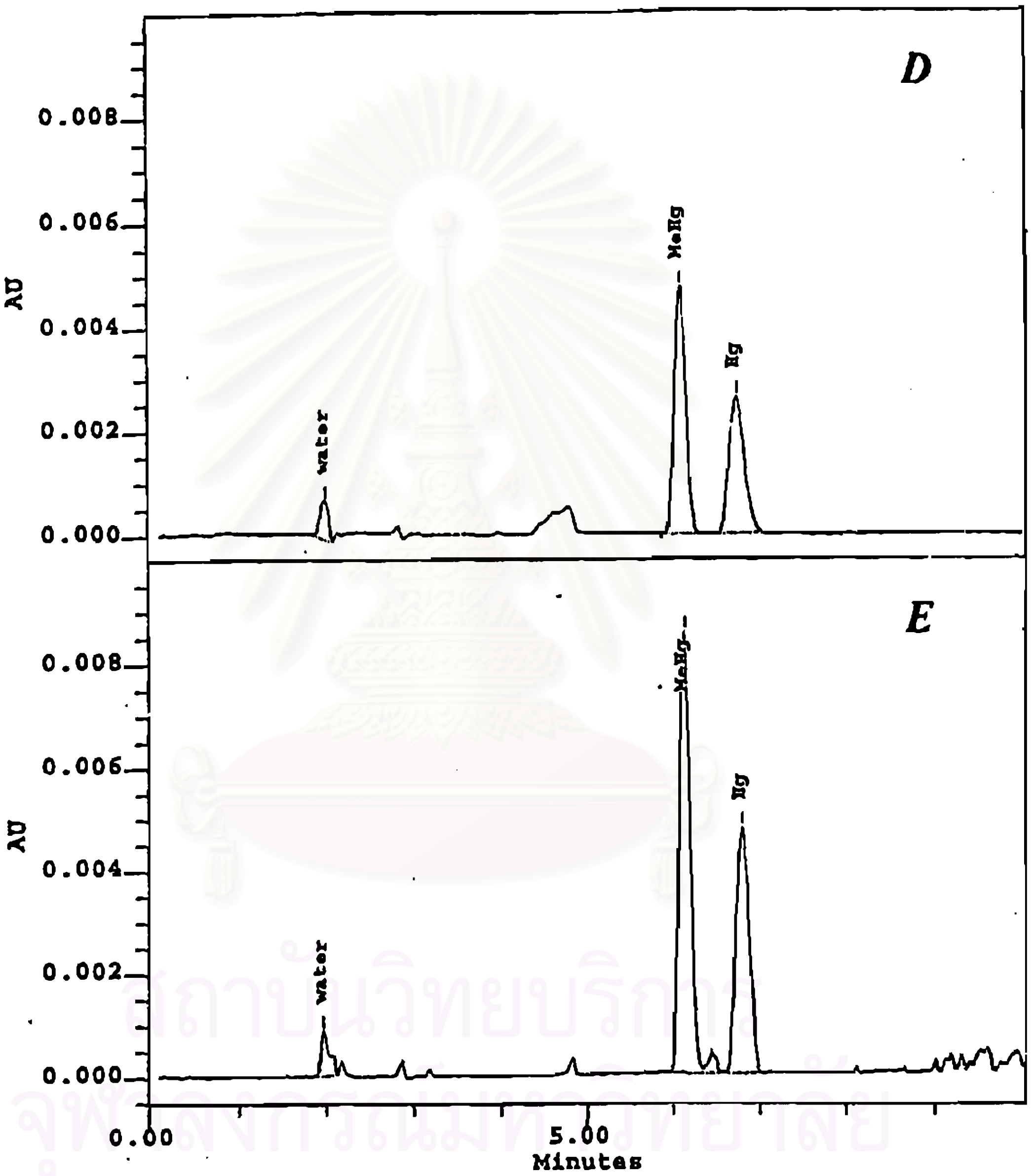

Figure 4.1.11 (continue)

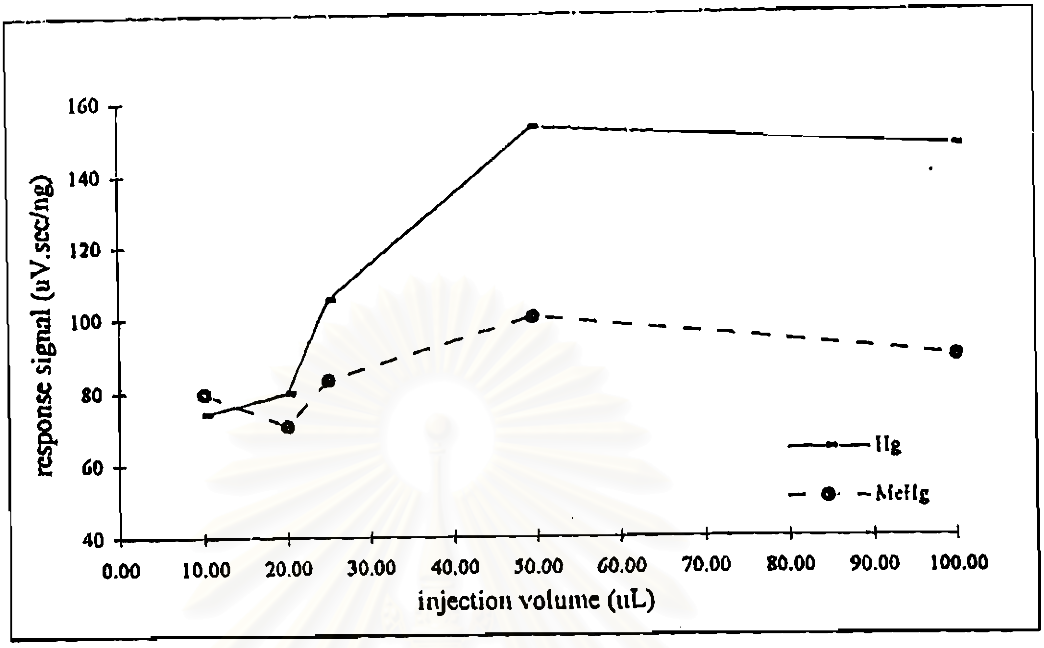

Figure 4.1.12: The relationship between the response signals (peak area) of inorganic and methylmercury and the injection volume in tetrabutylammonium bromide-2-mercaptoethanol system

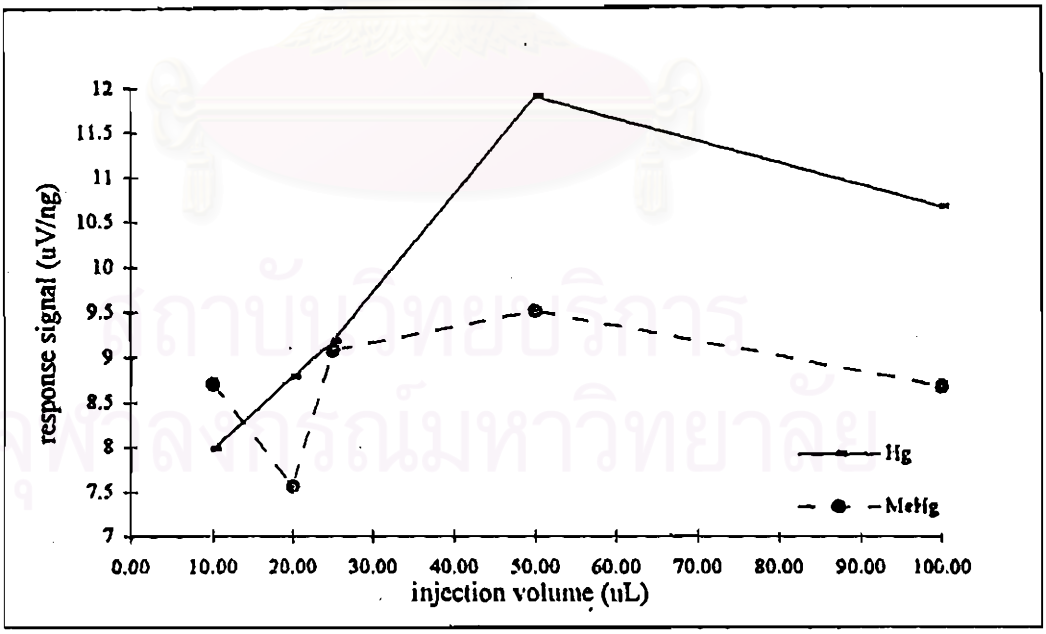

Figure 4.1.13: The relationship between the response signals (peak height) of inorganic and methylmercury and the injection volume in tetrabutylammonium bromide-2-mercaptoethanol system.

Form the studied of various effects on the resolution and sensitivity of the system the optimum conditions for the next study are shown in the Table 4.1.8.

Table 4.1.8: The optimum conditions of the tetrabutylammonium bromide-2-mercaptoethanol system

conditions	parameters
Column	Hypersil column, 250 x 4.6 mm.I.D., 5µm
Mobile phase composition	12:88% v/v of methanol-water buffered with 1.00×10^{-3} M acetate-acetic acid pH 3.00 containing 0.0075M TBABr and 0.0050%v/v 2-mercaptoethanol
Mobile phase flow rate	1.50 mL/min.
Injection volume	50 µL
Detector	Photodiode Array Detector
Data Acquisition	Maximum plot (200-400 nm)

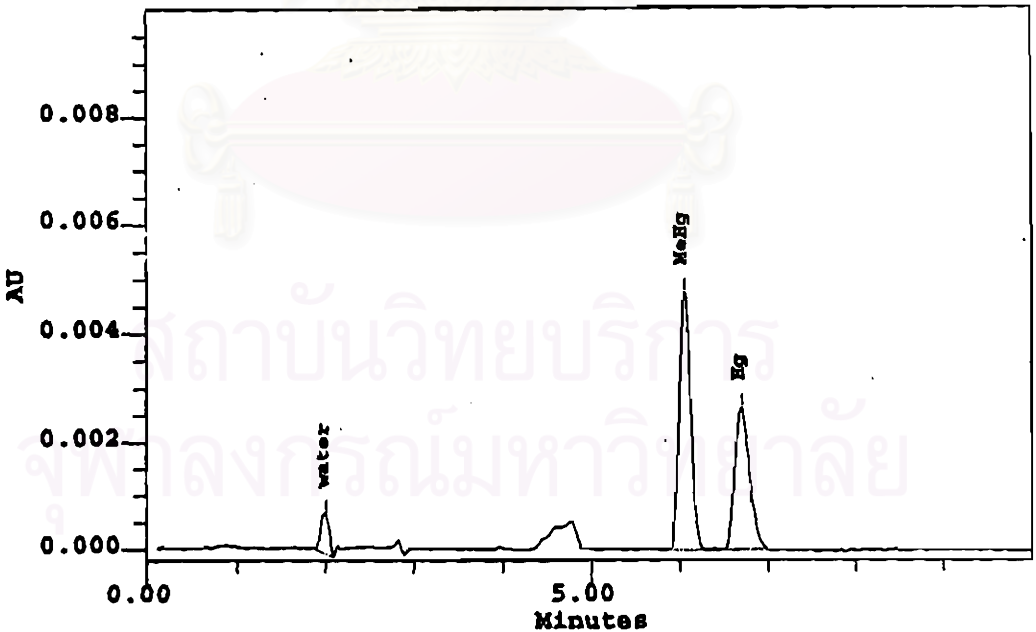

Figure 4.1.14: The chromatogram of inorganic and methylmercury under the optimum chromatographic conditions in tetrabutylammonium bromide-2-mercaptoethano system. (chromatographic conditions are shown in the Table 4.1.8)

4.1.3 The Study of Linearity of Standard Mercury Compounds

The linearity of standard mercury compounds, inorganic and methylmercury, are studied in the optimum chromatographic conditions as in Table 4.1.9 according to experimental section 3.4.3. The results of the study are shown in table 4.1.9. The relationships between peak area and peak height of each mercury compounds are plotted as the function of the concentration and are shown in Figures 4.1.15-4.1.18.

Table 4.1.9: The results of the study of linearity of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (replicate analyses, n=5)

Concentration (ppm)	Compound	Peak Area (μV.sec)	%RSD.	Peak Height (μV)	%RSD.
5.00	Hg^{2+}	23985	8.18	1750	8.79
	$MeHg^{+}$	28370	5.15	1890	3.83
10.00	Hg^{2+}	51920	5.33	3400	1.00
	$MeHg^{+}$	52201	13.60	3614	5.52
20.00	Hg^{2+}	128212	10.22	8005	3.38
	$MeHg^{+}$	120484	11.48	7571	5.02
50.00	Hg^{2+}	34596	2.10	17925	5.00
	$MeHg^{+}$	267659	3.19	20467	2.86
100.00	Hg^{2+}	763243	2.75	50372	5.22
	$MeHg^{+}$	605641	5.82	42093	1.05
200.00	Hg^{2+}	1576325	2.56	105406	2.35
	$MeHg^{+}$	1164508	3.51	78214	1.07
300.00	Hg^{2+}	2133872	0.36	122739	1.29
	$MeHg^{+}$	1751180	0.11	99598	0.32
400.00	Hg^{2+}	2840822	0.25	139042	2.57
	$MeHg^{+}$	2328816	0.66	118125	1.29
500.00	Hg^{2+}	3580434	0.98	192583	6.31
	$MeHg^{+}$	-	-	-	-

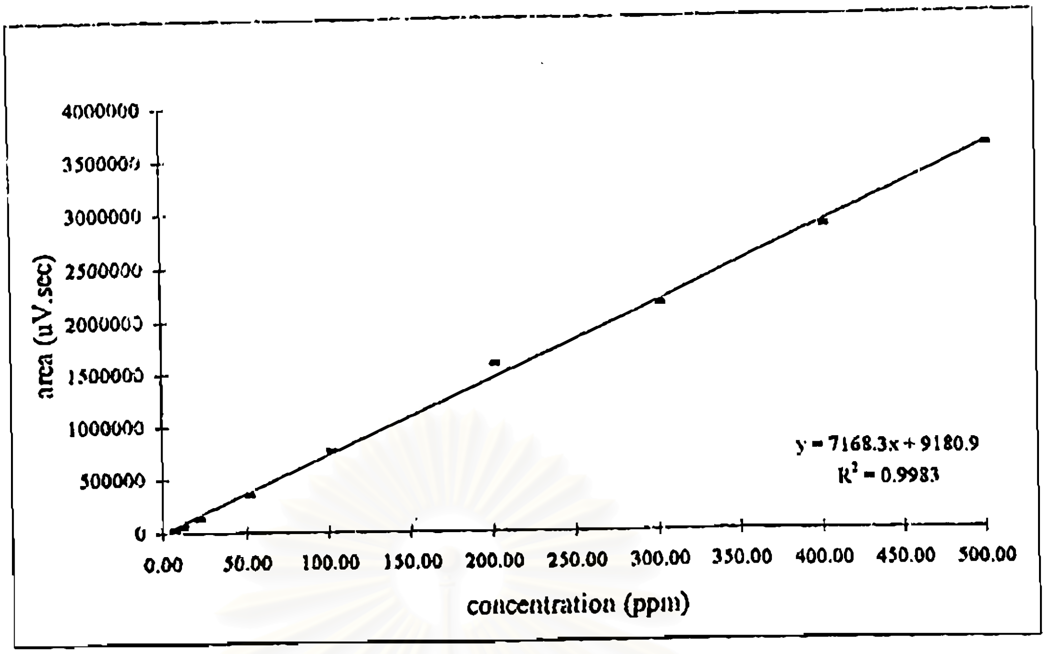

Figure 4.1.15: The relationship between the peak area of inorganic mercury and the concentration in tetrabutylammonium bromide-2-mercaptoethanol system.

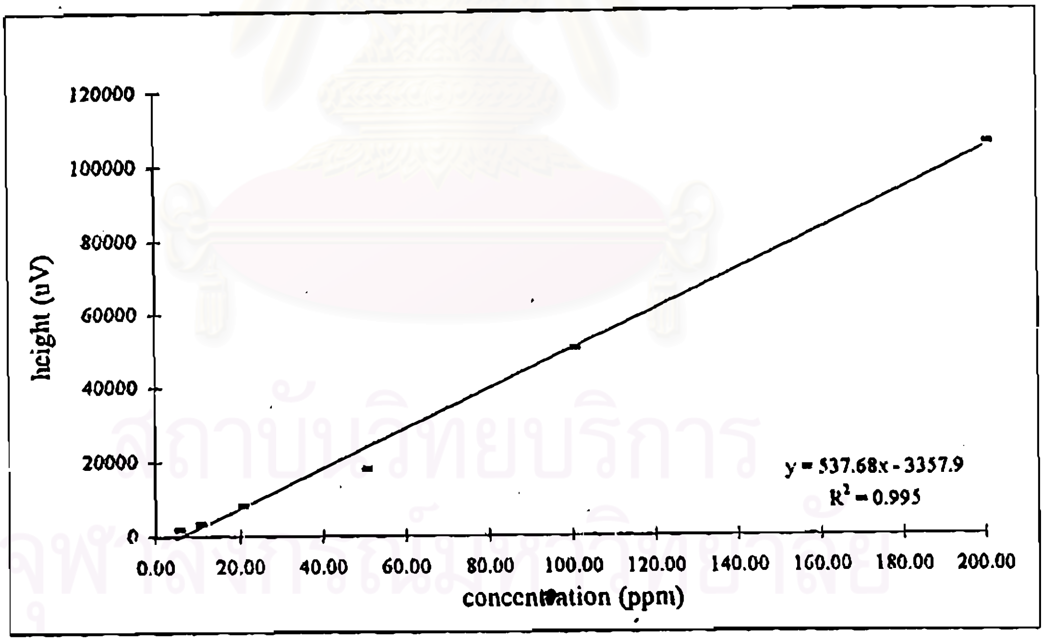

Figure 4.1.16: The relationship between the peak height of inorganic mercury and the concentration in tetrabutylammonium bromide-2-mercaptoethanol system.

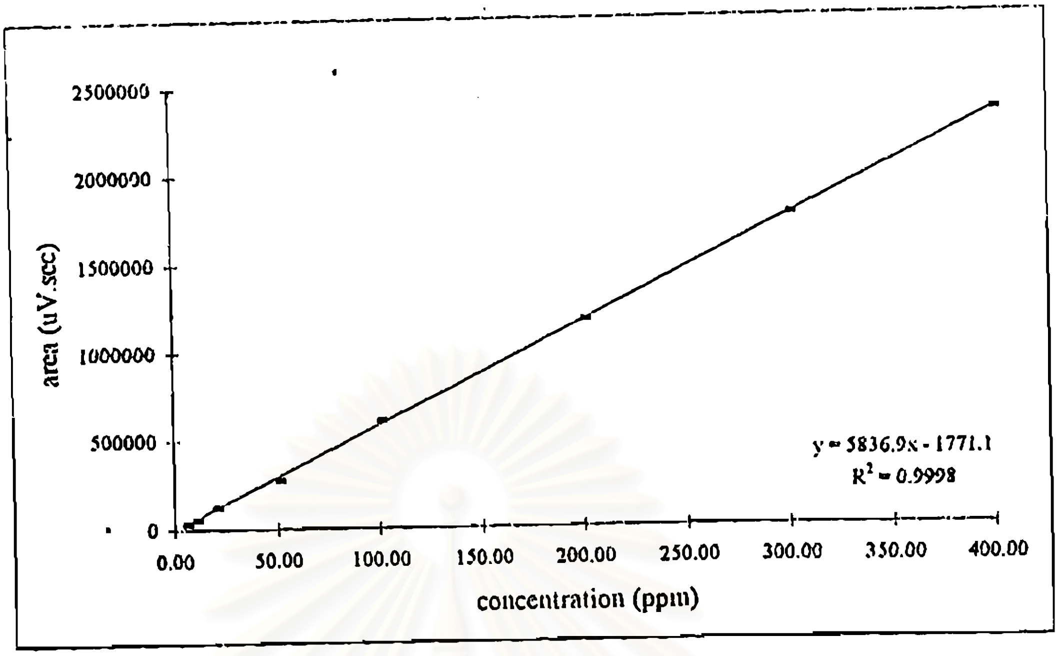

Figure 4.1.17: The relationship between the peak area of methylmercury and the concentration in tetrabutylammonium bromide-2-mercaptoethanol system.

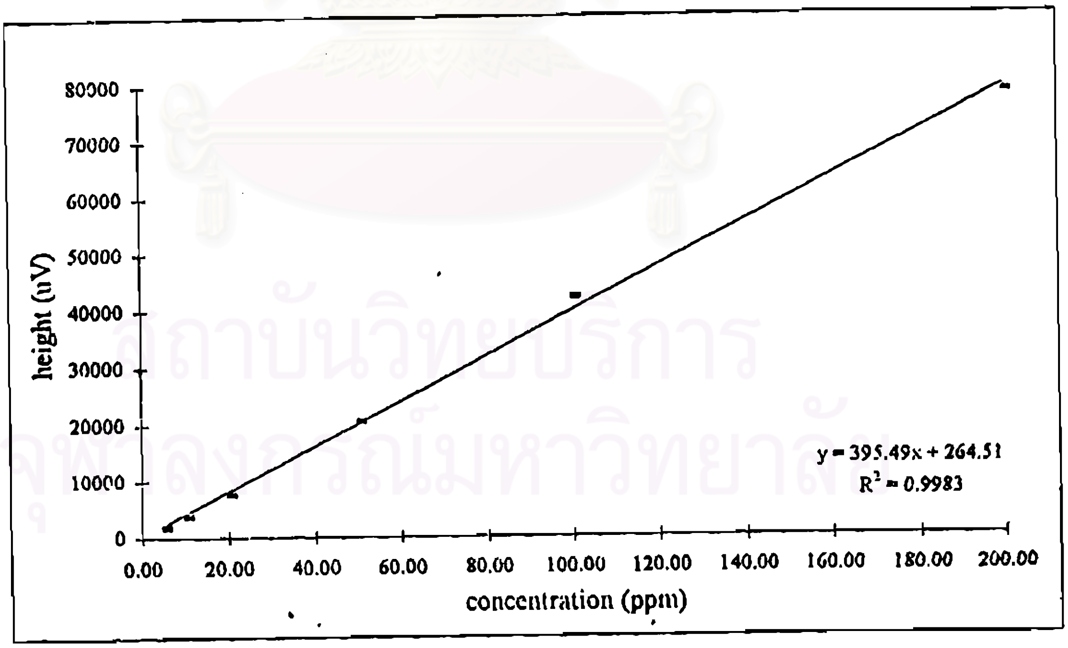

Figure 4.1.18: The relationship between the peak height of methylmercury and the concentration in tetrabutylammonium bromide-2-mercaptoethanol system.

From the Figures 4.1.15 and 4.1.17, the relationships between peak area and the concentration are plotted. The large linearity and good linear correlation of inorganic and methylmercury are obtained. The linearity over 500.00 ppm. and 400.00 ppm. are obtained for inorganic and methylmercury, respectively. However, the relationship between peak height and the concentration are shown in Figures 4.1.16 and 4.1.18. The same linearity over 200.00 ppm is obtained for both mercury compounds. However, the linearity of mercury compounds are not observed in higher concentration. From the study, peak area and peak height are obtained from the chromatogram but the smaller linearity and poorer linear coefficient are found from the peak height. Therefore, the only peak area is chosen for the next study. The concentrations in this linear range are chosen for the study of calibration curve.

4.1.4 The Study of Calibration Curve of Mercury Compounds

The large linear range of mercury compounds from the previous study are obtained. Six concentrations in the range 1.00-20.00 ppm are chosen for the study of calibration curve of mercury compounds at the optimum chromatographic condition in Table 4.1.9. according to the experimental section 3.4.4. The relationships between the peak area and the concentration of mercury compounds from Table 4.1.11 are plotted and shown in Figures 4.1.19-4.1.20. The percent relative standard deviation of mercury compounds in each concentration are also shown. The relative standard deviation of inorganic and methylmercuy are less than 3.50%, except in 1.00 and 3.00 ppm of methylmercury. However, the precision of the system is studied in the next section. From the calibration curves indicate that good linear correlation, $R^2 = 0.9995$ and 0.9976 are obtained for inorganic and methylmercury, respectively. The expression for inorganic mercury is $y = 9363.6x - 4750.3$ and methylmercury is $y = 6333.7x + 3623.7$. The higher sensitivity of inorganic mercury is found from comparison the slope of mercury compounds.

Table 4.1.10: The results of the study of calibration curve of inorganic and methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system. (replicate analyses, n=5)

Concentration (ppm)	Compound	Peak Area (μV.sec)	%RSD.
1.00	Hg^{2+}	6895	2.63
	$MeHg^{+}$	5575	12.64
3.00	Hg^{2+}	21288	1.82
	$MeHg^{+}$	12758	7.52
6.00	Hg^{2+}	51615	2.32
	$MeHg^{+}$	32935	2.55
10.00	Hg^{2+}	87615	0.78
	$MeHg^{+}$	61655	3.27
15.00	Hg^{2+}	136040	0.86
	$MeHg^{+}$	89559	2.13
20.00	Hg^{2+}	183044	0.58
	$MeHg^{+}$	124129	2.61

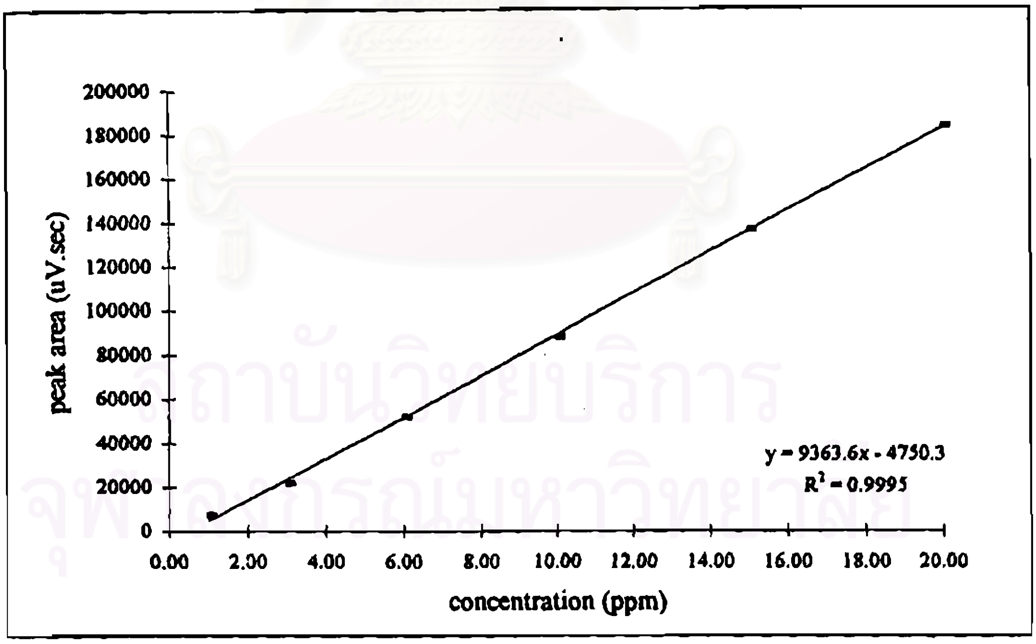

Figure 4.1.19 : The calibration curve of inorganic mercury in tetrabutylammonium bromide-2-mercaptoethanol system.

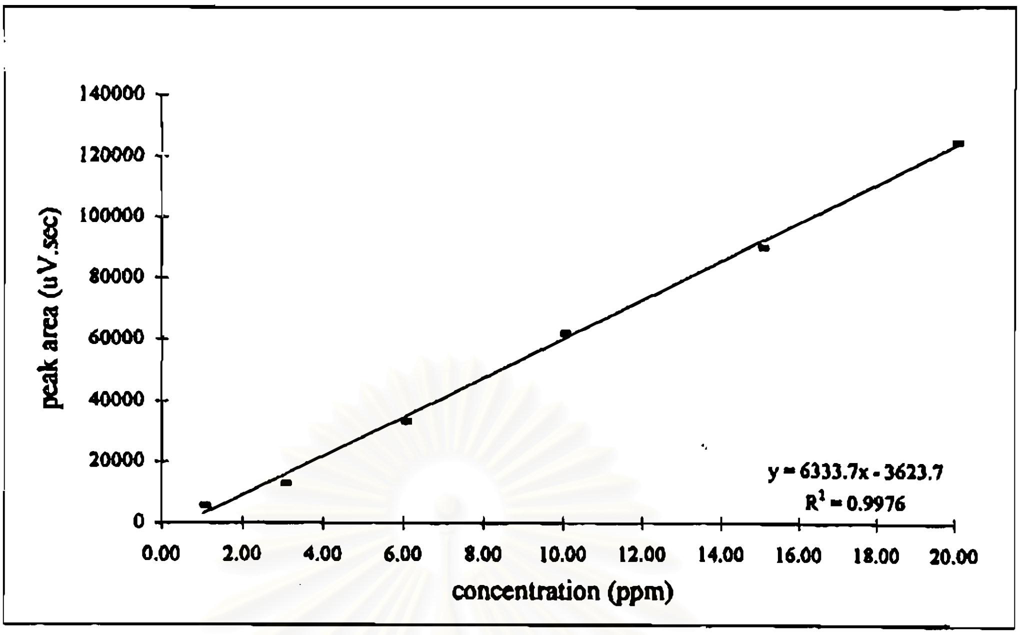

Figure 4.1.20 : The calibration curve of methylmercury in tetrabutylammonium bromide-2-mercaptoethanol system.

4.1.5 The Study of Detection Limit of the System

The detection limit of mercury compounds in tetrabutylammonium bromide-2-mercaptoethanol system are studied according to the experimental section 3.4.5. The optimum chromatographic conditions for the study are shown in Table 4.1.9. From the study the same detection limits as 0.3 ppm (as Hg) for 50 μL injection volume (15 ng as Hg) is found for both mercury compounds. The detection limit of mercury compounds in this system will compare with the detection limit of hexanesulfonate-2-mercaptoethanol system and are presented in Table 4.2.12

4.1.6 The Study of Precision of the System

The precision of mercury compounds for tetrabutylammonium bromide-2-mercaptoethanol system are studied according to the experimental section 3.4.6 and the AOAC® Peer-Verified Methods Program (87). The repeatability of the system is determined by fifth injection of a series standards mercury compounds. The standards are contained 4.00, 8.00, 12.00, and 16.00 ppm. The percent relative standard deviation from the study are presented in Table 4.1.11 The results show that the percent relative standard deviations of all concentration in the calibration curve are less than the acceptability percent relative standard deviation from the AOAC method. Inorganic mercury gives less deviation than methylmercury in the concentration 8.00 and 12.00 ppm, but in high and low concentration methylmercury give less deviation. In low concentration (4.00 ppm) both inorganic and methylmercury give high deviation; however, the percent relative standard deviation of both are less than in the AOAC method. It is shown that the precision of the method is acceptable.

Table 4.1.11: The result of the study of precision for the tetrabutylammonium bromide-2-mercaptoethanol system compare with the acceptable percent relative standard deviation of the reference method

concentration	Hg^{2+}		$MeHg^{+}$		AOAC method
(ppm)	peak area (μV.sec)	%RSD	peak area (μV.sec)	%RSD	%RSD
4.00	50044 47852 47831 54192 55559	7.04	22606 20709 24321 22457 23942	6.25	8.71
8.00	89936 87502 89740 90883 89168	1.40	49101 49404 51969 48477 50415	2.74	7.85
12.00	159617 160853 166603 166377 165366	2.00	71550 73459 69670 74990 75366	3.28	7.37
16.00	222195 204031 208260 219640 222975	4.10	90901 91042 90654 89494 95205	2.38	7.07

4.2 The Study of Sodium Hexanesulfonate-2-Mercaptoethanol System

4.2.1 The Study of Various Effects on the Resolution of Mercury Compounds

Various effects on the resolution of mercury compounds, i.e. the mobile phase pH, 2-mercaptoethanol concentration, sodium hexanesulfonate concentration methanol composition in mobile phase, and mobile phase flow rate are studied. The mobile phase compose of methanol-water (30:70% v/v) gives long retention time for phenylmercury detection. When percent methanol in mobile phase is increased to 40:60% v/v, the retention time of phenylmercury is decreased and the resolution between inorganic and methylmercury is acceptable. Thus, in this mobile phase system the mobile phase composed of methanol-water (40:60% v/v) is used for the studies.

4.2.1.1 The Effect of Mobile Phase pH

The mobile phase pH is studied to find the optimum pH that the mixed complexing agent and ion-pairing agent can be form complex or ion pair appositely with the mercury compounds as in the tetrabutylammonium bromide-2-mercaptoethanol system. The mobile phase pH in the range 2.0-8.0 adjusted with hydrochloric acid and sodium hydroxide solution. is preliminary studied to find the optimum mobile phase pH. From the preliminary study, pH range 3.00-6.00 is chosen for the next studies. The mixture of methanol-water (40:60 % v/v) containing 0.0050% v/v of 2-mercaptoethanol and 0.0050M of sodium hexanesulfonate buffered with acetate-acetic acid buffer solution is used for the mobile phase in this study. The mobile phase pH in range 3.00-6.00 is studied according to experimental section 3.5.1.1 and Table 3.2.1. The effects of mobile phase pH on the resolution of mercury compounds are presented in Table 4.2.1 and Figure 4.2.1. From varied pH 3.00 to 6.00, asymmetric peaks as tailing of inorganic mercury are observed, but the more symmetrical peaks are found in pH 4.00 -5.00. The slightly-tailing peaks of methylmercury are found in all of pH except in pH 5.00. The highest resolution of inorganic and methylmercury is also found in pH 5.00. Therefore, the pH 5.00 is chosen as the optimum mobile phase pH for the next study.

Table 4.2.1: The results of the effect of mobile phase pH on the chromatographic parameters for the separation of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system. (Triplicate analyses)

pH	compound	t_R (min)	W (min)	k'	α*	R_S**
3.00	solvent	2.829±0.013	-	-		
	Hg^{2+}	4.200±0.009	0.333±0.016	0.485	1.348	1.129
	$MeHg^+$	4.680±0.006	0.517±0.070	0.654		
	$PhHg^+$	15.429±0.057	0.861±0.135	4.454		
4.00	solvent	2.871±0.003	-	-		
	Hg^{2+}	4.067±0.057	0.323±0.038	0.416	1.445	1.554
	$MeHg^+$	4.597±0.022	0.360±0.019	0.601		
	$PhHg^+$	14.464±0.230	0.663±0.104	4.038		
4.50	solvent	2.832±0.007	-	-		
	Hg^{2+}	4.194±0.005	0.321±0.086	0.481	1.351	1.297
	$MeHg^+$	4.674±0.007	0.420±0.054	0.650		
	$PhHg^+$	15.371±0084	0.883±0.081	4.428		
5.00	solvent	2.825±0.004	-	-		
	Hg^{2+}	4.141±0.011	0.250±0.035	0.466	1.382	1.794
	$MeHg^+$	4.645±0.006	0.312±0.028	0.644		
	$PhHg^+$	14.974±0.057	0.762±0.167	4.300		
6.00	solvent	2.826±0.005	-	-		
	Hg^{2+}	4.156±0.015	0.397±0.081	0.471	1.408	1.341
	$MeHg^+$	4.699±0.006	0.413±0.059	0.663		
	$PhHg^+$	15.650±0.022	0.713±0.090	4.538		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

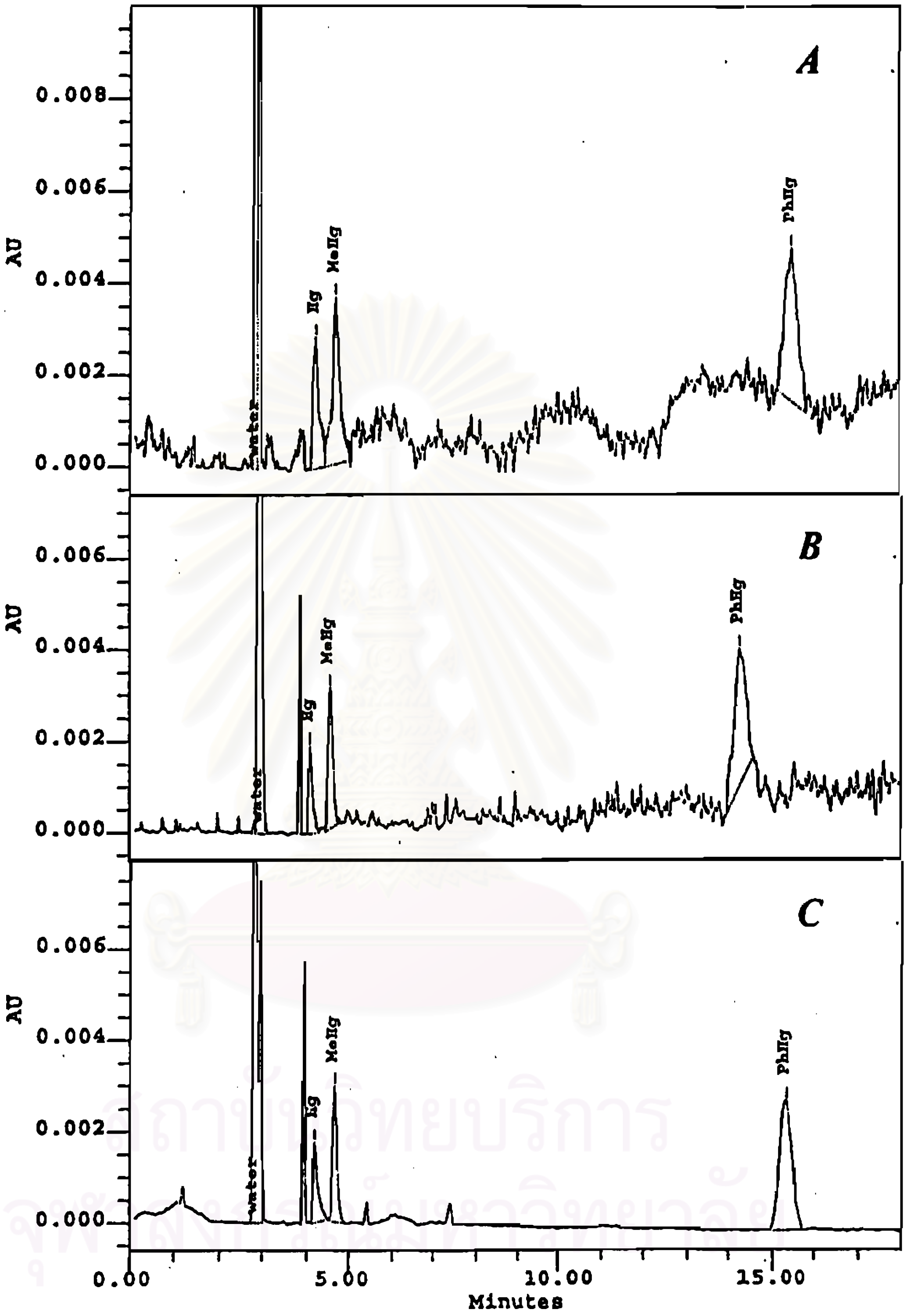

Figure 4.2.1: The chromatograms of mercury compounds at variety mobile phase pH adjusted with acetate acetic acid buffer in sodium hexanesulfonate-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.2.1)

A) 3.00 B) 4.00 C) 4.50 D) 5.00 E) 6.00

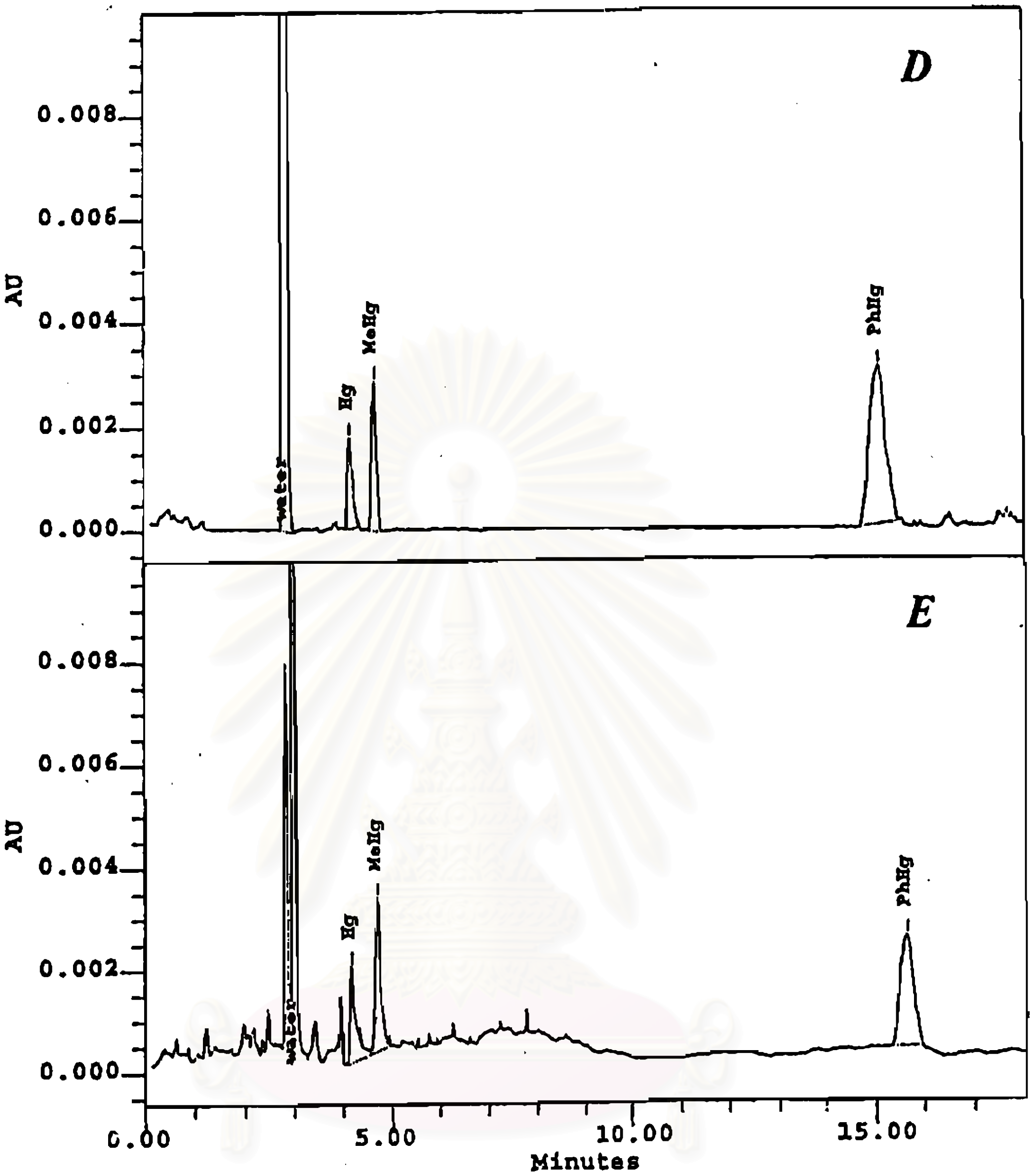

Figure 4.2.1 (continue)

4.2.1.2 The Effect of Acetate-Acetic Acid Buffer Solution

The acetate-acetic acid buffer solution is used to control the mobile phase pH. The buffer concentrations 1.00 x 10^{-3}, 2.00 x 10^{-3}, and 5.00 x 10^{-3} M in mobile phase composed of mixture methanol-water (40:60% v/v), 0.0050M of sodium hexanesulfonate, and 0.0050% v/v of 2-mercaptoethanol are studied according to experimental section 3.5.1.2 and Table 3.2.1. The effects of mobile phase pH in this system are as same as the previous system. It is found that the peak resolution and peak shape of mercury compounds are not effected from the increasing buffer concentration, but the baseline signals are effected. Therefore, the acetate-acetic acid buffer concentration 1.00 x 10^{-3} M is chosen as the optimum concentration for the next study.

4.2.1.3 The Effect of 2-Mercaptoethanol Concentration

2-Mercaptoethanol is used as complexing agent as in the previous system. The effects of 2-mercaptoethanol concentration on the resolution of mercury compounds according to experimental section 3.5.1.3 are studied. The mixtures of methanol-water (40:60% v/v) buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer solution pH 5.00 containing 0.0050M sodium hexanesulfonate and the variety of 2-mercaptoethanol concentration are used as mobile phase in this study. The chromatographic conditions are shown in Table 3.2.2 The 2-mercaptoethanol concentrations in this study are varied from 0.0020% v/v to 0.0080%v/v. The effects of 2-mercaptoethanol concentration are presented in Table 4.2.2 and Figure 4.2.2. In the concentration 0.0020% v/v, inorganic and methylmercury are incompletely separated and asymmetric peaks of mercury compounds are found. When 2-mercaptoethanol concentration is increased, more symmetric peaks are observed. Inorganic and methylmercury are completely separated. In 0.0040% v/v of 2-mercaptoethanol, the lowest concentration that mercury compounds are completely separated, the symmetrical methylmercury peak is observed, but the asymmetric peaks of inorganic and phenylmercury are still found. The resolution is increased with increasing 2-mercaptoethanol concentration, but noisy signal is also found. Although in

0.0040% v/v of 2-mercaptoethanol the resolution is lower than in higher concentration, this concentration the baseline signal is not effected. It is found that the optimum resolution and peak shape are obtained in 0.0040% v/v 2-mercaptoethanol. Therefore, the 0.0040% v/v of 2-mercaptoethanol is chosen for the next study.

Table 4.2.2: The results of the effect of 2-mercaptoethanol concentration on the chromatographic parameters for the separation of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system.. (Triplicate analyses)

%mercapto ethanol	compound	t_R (min)	W (min)	k'	α*	R_s**
0.0020%	solvent	2.962±0.026	-	-		
	Hg^{2+}	4.169±0.011	0.473±0.043	0.407	1.410	1.100
	$MeHg^+$	4.662±0.014	0.423±0.015	0.574		
	$PhHg^+$	15.125±0.139	1.138±0.172	4.106		
0.0040%	solvent	2.939±0.066	-	-		
	Hg^{2+}	4.195±0.008	0.407±0.032	0.427	1.389	1.194
	$MeHg^+$	4.682±0.012	0.410±0.056	0.593		
	$PhHg^+$	15.442±0.102	0.8930.052	4.254		
0.0060%	solvent	2.858±0.051	-	-		
	Hg^{2+}	4.177±0.062	0.421±0.066	0.461	1.401	1.301
	$MeHg^+$	4.704±0.008	0.390±0.042	0.646		
	$PhHg^+$	15.704±0.051	0.917±0.062	4.495		
0.0080%	solvent	2.819±0.007	-	-		
	Hg^{2+}	4.222±0.009	0.294±0.070	0.498	1.357	1.417
	$MeHg^+$	4.725±0.010	0.416±0.072	0.676		
	$PhHg^+$	15.995±0.140	0.780±0.098	4.674		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

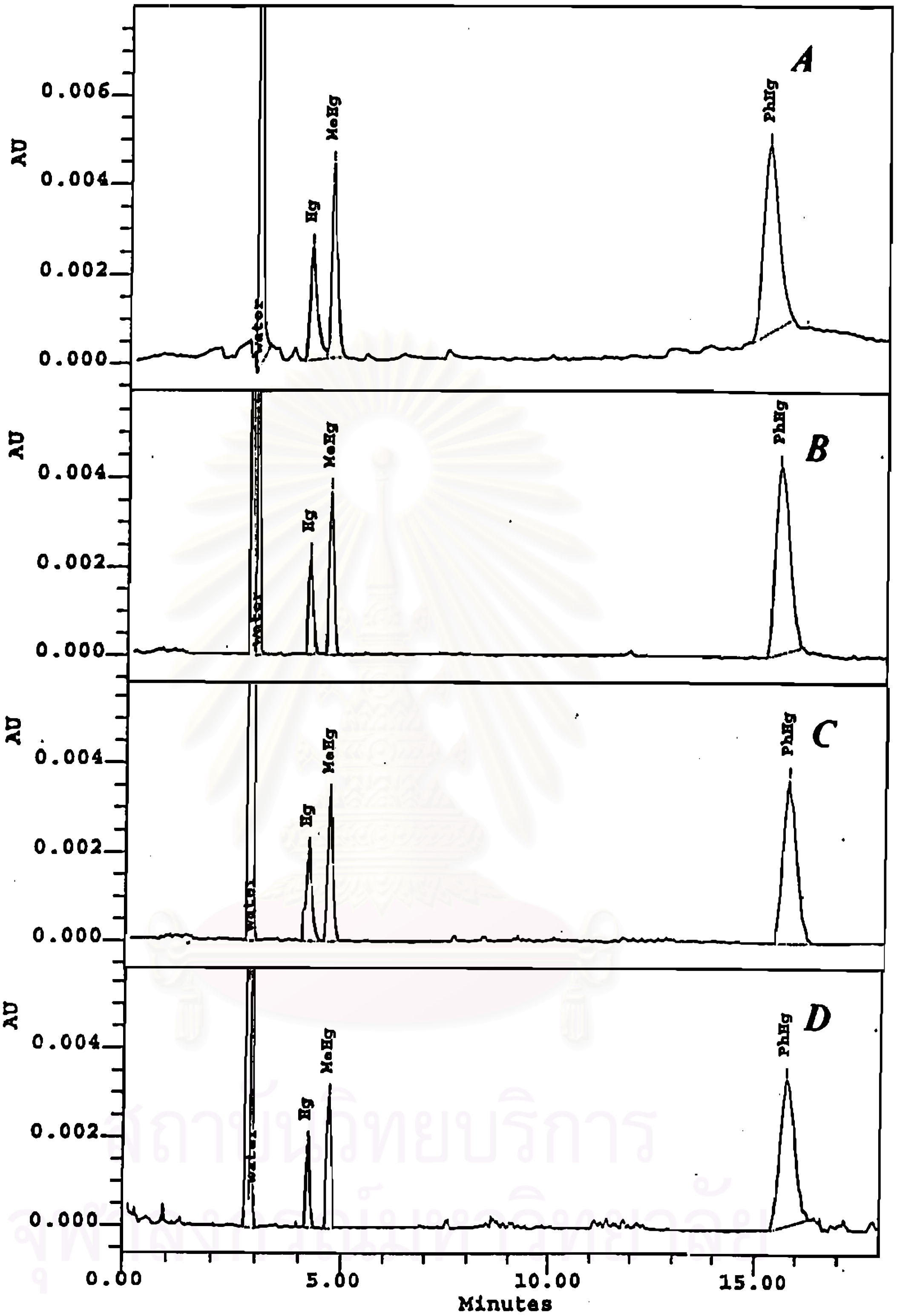

Figure 4.2.2: The chromatograms of mercury compounds at variety 2-mercaptoethanol concentration in sodium hexanesulfonate-2-mercaptoethanol system.(chromatographic conditions are shown in Table 3.2.3)

A) 0.0020% B) 0.0040% C) 0.0060% D) 0.0080%

4.2.1.4 The Effect of Sodium Hexanesulfonate Concentration

Form the previous studies, the optimum mobile phase conditions are performed as the mixture of methanol-water (40:60% v/v) buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 5.00 containing 0.0040% v/v of 2-mercaptoethanol. In this study optimum concentration of sodium hexanesulfonate, the anion-pairing agent, is found. Sodium hexanesulfonate concentrations 0.0000-0.0150M are studied according to experimental section 3.5.1.4 and Table 3.2.4. The effects of sodium hexanesulfonate concentration on the resolution of mercury compounds are presented in Table 4.2.3 and Figure 4.2.3.

In the absence of sodium hexanesulfonate in mobile phase, the resolution between inorganic and methylmercury is less than in the presence of sodium hexanesulfonate. When sodium hexanesulfonate is added to the mobile phase (0.0025-0.0040M), the resolutions are increased and the more symmetric peak of mercury compounds are found. When sodium hexanesulfonate concentrations are increased to higher concentrations (0.0060-0.0150M), the asymmetric peaks are found again. At concentrations 0.0025M and 0.0040M, the resolutions and peak shapes of mercury compound are not different. However, at 0.0025M the column equilibration time is long. At higher sodium hexanesulfonate the equilibration time is reduced. And in 0.0040M the more reproducibility of peak shape and peak width are found. It is found that the optimum resolution and peak shapes are obtained in 0.0040M sodium hexanesulfonate. Therefore, the 0.0040M sodium hexanesulfonate is chosen for the next study.

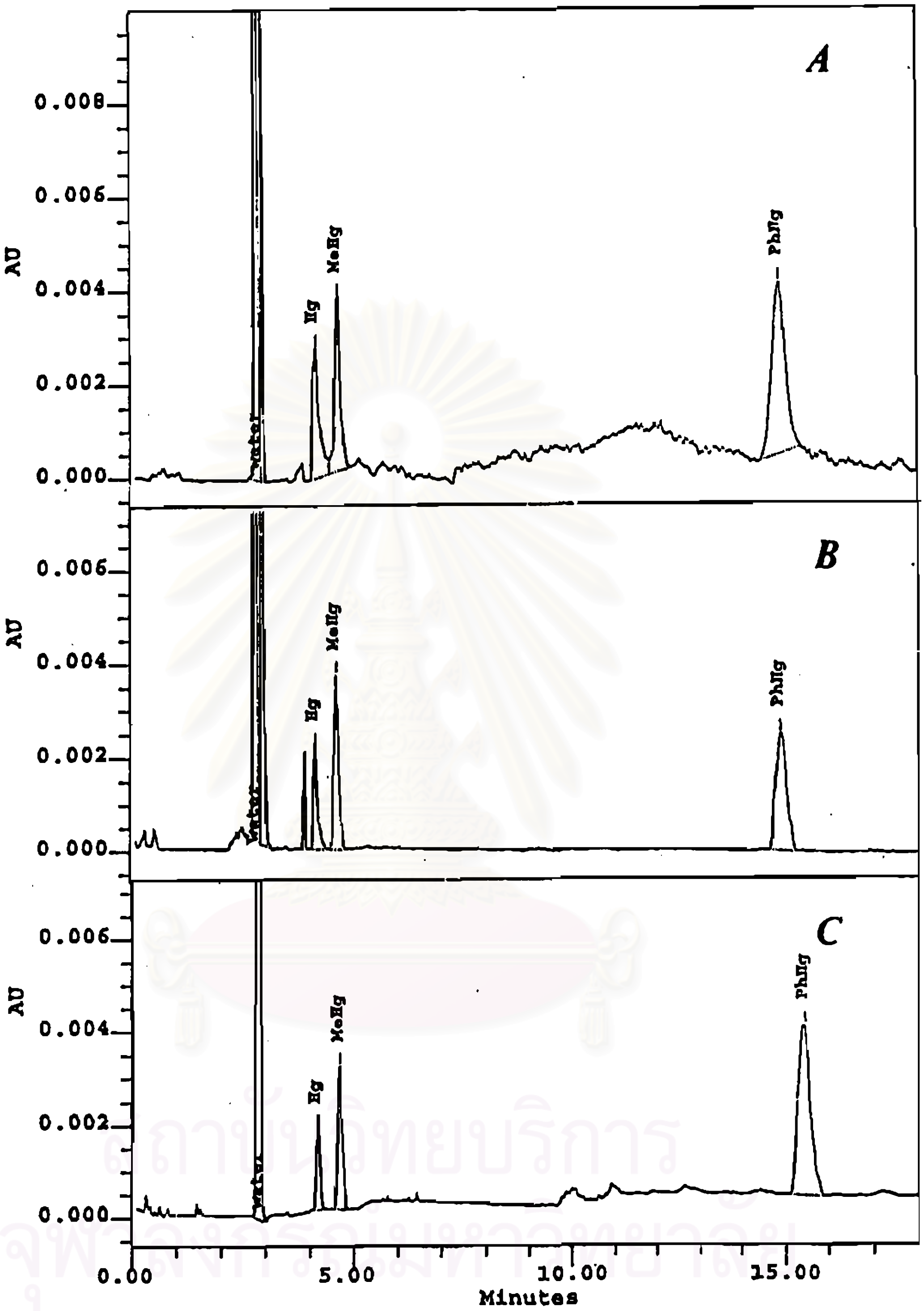

Figure 4.2.3: The chromatograms of mercury compounds at variety sodium hexanesulfonate concentration in sodium hexanesulfonate-2-mercaptoethanol system.(chromatographic conditions are shown in Table 3.2.4)

A) 0.0000M B) 0.0010M C) 0.0025M

D) 0.0040M E) 0.0075M F) 0.0150M

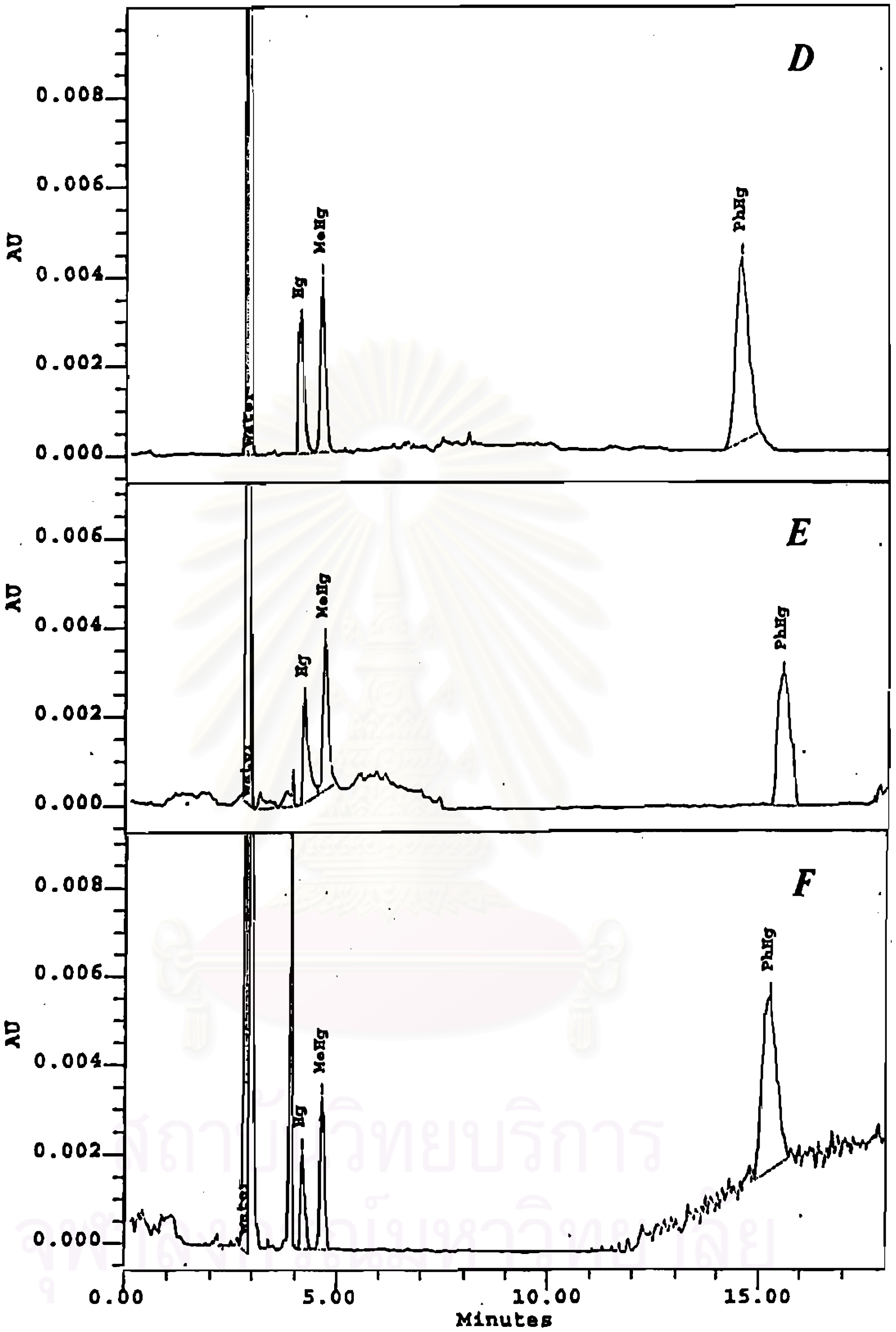

Figure 4.2.3 (continue)

Table 4.2.3: The results of the effect of sodium hexanesulfonate concentration on the chromatographic parameters for the separation of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system. (Triplicate analyses)

hexanesulfonated concentration	compound	t_R (min)	W (min)	k'	α*	R_s**
0.0000M	solvent	2.827±0.006	-	-		
	Hg^{2+}	4.140±0.012	0.457±0.052	0.464	1.384	1.096
	$MeHg^+$	4.643±0.005	0.462±0.025	0.642		
	$PhHg^+$	14.870±0.058	0.957±0.048	4.260		
0.0010M	solvent	2.823±0.006	-	-		
	Hg^{2+}	4.156±0.005	0.407±0.067	0.472	1.367	1.165
	$MeHg^+$	4.643±0.010	0.429±0.060	0.645		
	$PhHg^+$	14.813±0.164	0.777±0.228	4.247		
0.0025M	solvent	2.867±0.024	-	-		
	Hg^{2+}	4.186±0.008	0.358±0.087	0.460	1.380	1.376
	$MeHg^+$	4.687±0.007	0.371±0.069	0.635		
	$PhHg^+$	15.382±0.195	0.854±0.055	4.365		
0.0040M	solvent	2.838±0.0037	-	-		
	Hg^{2+}	4.093±0.004	0.466±0.029	0.442	1.407	1.109
	$MeHg^+$	4.603±0.008	0.454±0.020	0.622		
	$PhHg^+$	14.523±0.040	0.833±0.023	4.117		
0.0050M	solvent	2.903±0.046	-	-		
	Hg^{2+}	4.196±0.009	0.333±0.081	0.445	1.362	1.259
	$MeHg^+$	4.663±0.023	0.408±0.029	0.606		
	$PhHg^+$	15.163±0.307	0.917±0.072	4.223		
0.0075M	solvent	2.879±0.012	-	-		
	Hg^{2+}	4.196±0.010	0.395±0.035	0.457	1.361	1.145
	$MeHg^+$	4.670±0.033	0.433±0.033	0.622		
	$PhHg^+$	15.446±0.294	0.825±0.129	4.365		
0.0100M	solvent	2.894±0.078	-	-		
	Hg^{2+}	4.140±0.003	0.462±0.044	0.430	1.419	1.153
	$MeHg^+$	4.660±0.029	0.440±0.061	0.610		
	$PhHg^+$	15.177±0.337	0.933±0.104	4.244		
0.0150M	solvent	2.860±0.058	-	-		
	Hg^{2+}	4.167±0.010	0.399±0.023	0.457	1.365	1.184
	$MeHg^+$	4.644±0.023	0.407±0.121	0.624		
	$PhHg^+$	15.077±0.306	0.804±0.082	4.272		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

4.2.1.5 The Effect of the Methanol Composition in Mobile phase

The methanol composition in mobile phase is greatly affected to the retention time and peak resolution of mercury compounds as found in the previous system. The mixture of methanol-water buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 5.00 containing 0.0040M sodium hexanesulfonate and 0.0040% v/v 2-mercaptoethanol is used as the mobile phase for the study according to experimental section 3.5.1.5 and Table 3.2.5. The effects of methanol composition in mobile phase compounds are presented in Table 4.2.4. and Figure 4.2.4-4.2.6. As in the tetrabutylammonium bromide-2-mercaptoethanol system, the low proportions of methanol give high resolution and long retention time. In low proportion of methanol (10-20%), the phenylmercury peak is not detected. The increasing proportion of methanol the resolution and retention time are decreased, until 70% methanol inorganic and methylmercury are not separated. The different from previous system is that in low proportion of methanol (10%) the resolution of inorganic and methylmercury is zero. It is found that the optimum retention time, peak resolution, and peak shape are obtained in methanol 40% in mobile phase. Therefore, the mobile phase composed of 40% methanol is chosen for the next study.

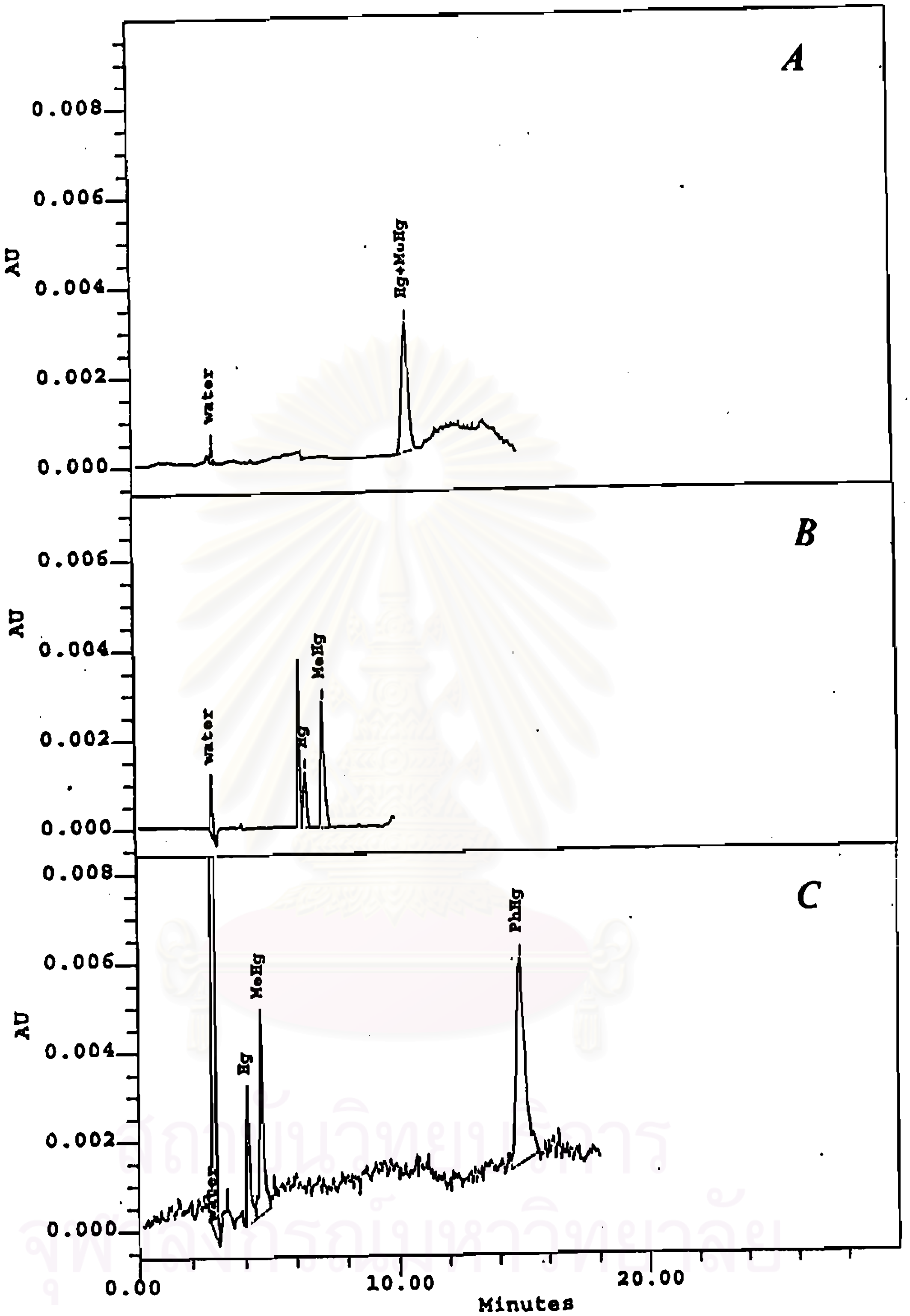

Figure 4.2.4: The chromatograms of mercury compounds at variety methanol composition in mobile phase in sodium hexanesulfonate-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.2.5)

A) 10% B) 20% C) 40% D) 50% E) 60% H) 70%

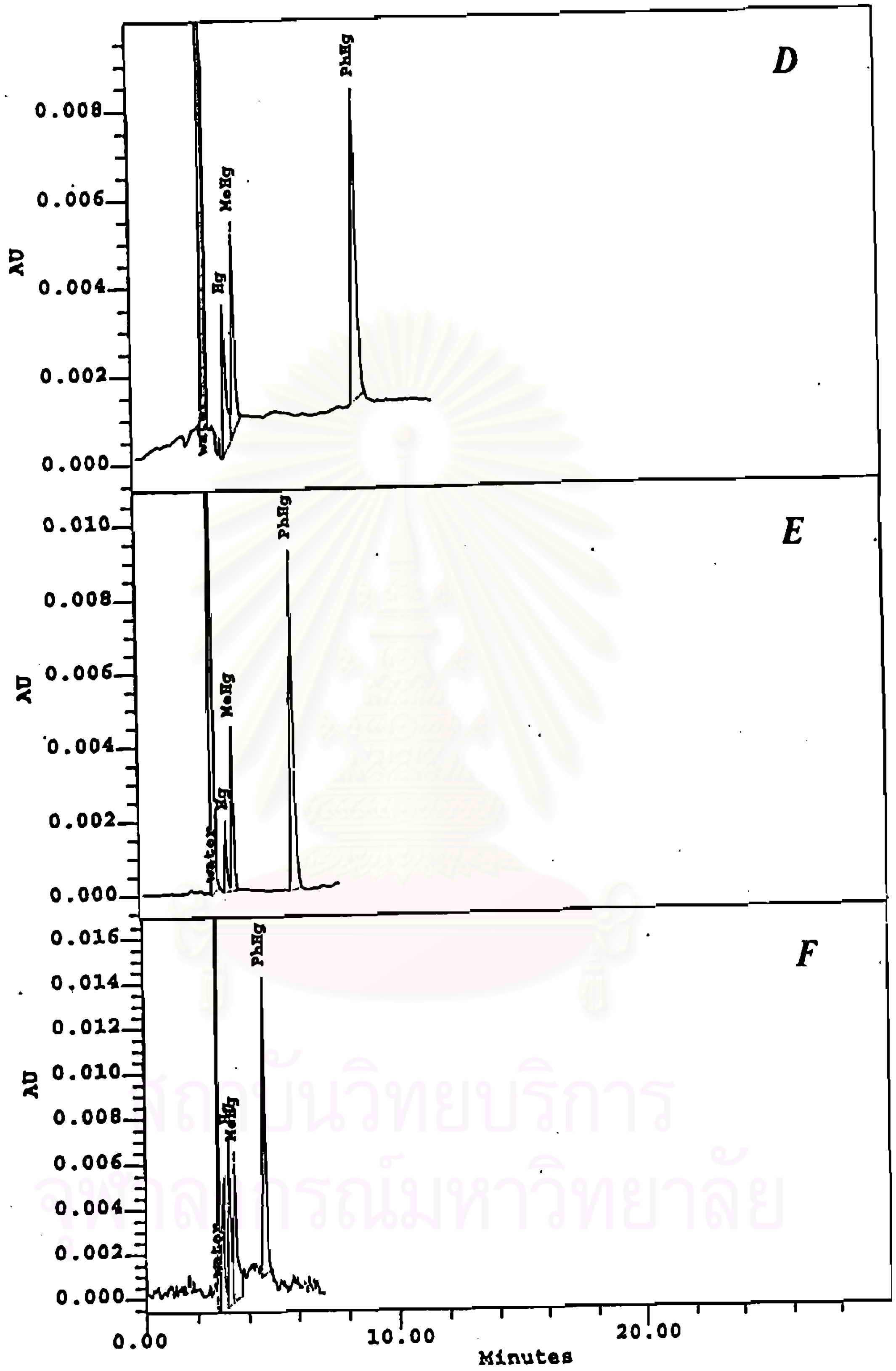

Figure 4.2.4 (continue)

Table 4.2.4: The results of the effect of the methanol composition in mobile phase on the chromatographic parameters for the separation of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system.. (Triplicate analyses)

%MeOH	compound	t_R (min)	W (min)	k'	α*	R_S**
10	solvent	3.000±0.050	-	-		
	Hg^{2+}	10.587±0.031	0.733±0.083	2.529	1.000	0.000
	$MeHg^{+}$					
20	solvent	2.906±0.006	-	-		
	Hg^{2+}	6.516±0.022	0.333±0.047	1.242	1.188	1.817
	$MeHg^{+}$	7.192±0.018	0.412±0.080	1.475		
30	solvent	2.853±0.003	-	-		
	Hg^{2+}	5.040±0.010	0.425±0.081	0.766	1.261	1.414
	$MeHg^{+}$	5.610±0.008	0.381±0.050	0.966		
	$PhHg^{+}$	28.097±0.048	1.554±0.129	8.848		
40	solvent	2.827±0.006	-	-		
	Hg^{2+}	4.131±0.022	0.300±0.033	0.461	1.386	1.245
	$MeHg^{+}$	4.634±0.005	0.508±0.017	0.639		
	$PhHg^{+}$	14.871±0.033	0.843±0.061	4.260		
50	solvent	2.922±0.040	-	-		
	Hg^{2+}	3.689±0.025	0.313±0.008	0.262	1.511	1.046
	$MeHg^{+}$	4.079±0.003	0.433±0.056	0.396		
	$PhHg^{+}$	9.069±0.038	0.70±70.074	2.104		
60	solvent	2.867±0.026	-	-		
	Hg^{2+}	3.401±0.039	0.313±0.028	0.186	1.607	0.944
	$MeHg^{+}$	3.724±0.005	0.370±0.007	0.299		
	$PhHg^{+}$	6.154±0.010	0.620±0.040	1.146		
70	solvent	2.939±0.024	-	-		
	Hg^{2+}	3.461±0.001	0.417±0.016	0.178	1.000	0.000
	$MeHg^{+}$					
	$PhHg^{+}$	4.633±0.009	0.506±0.117	0.576		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

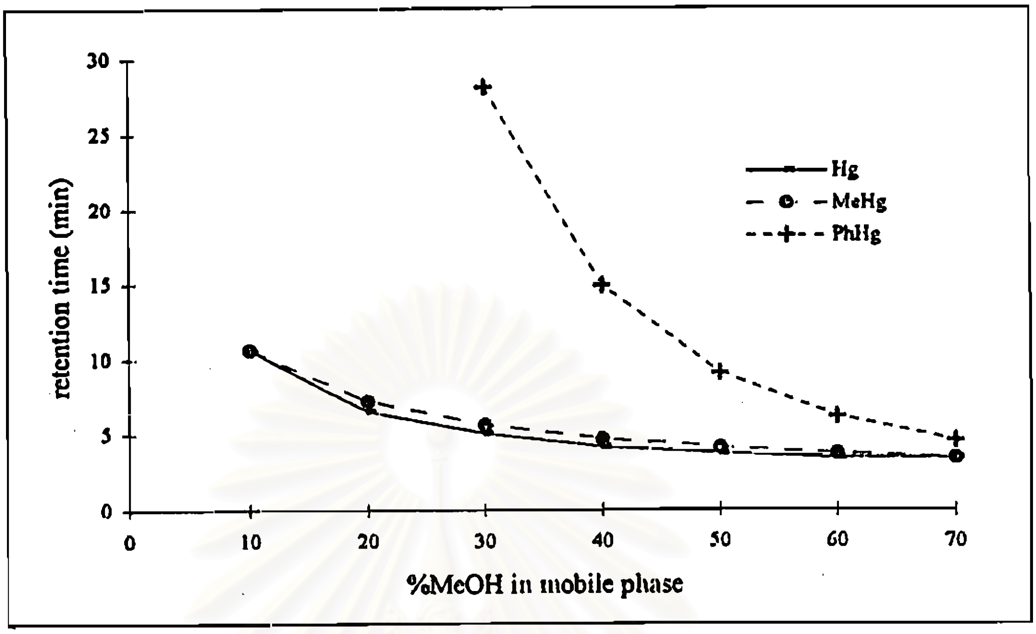

Figure 4.2.5: The relationship between the retention time of mercury compounds and percent methanol in mobile phase in sodium hexanesulfonate-2-mercaptoethanol system.

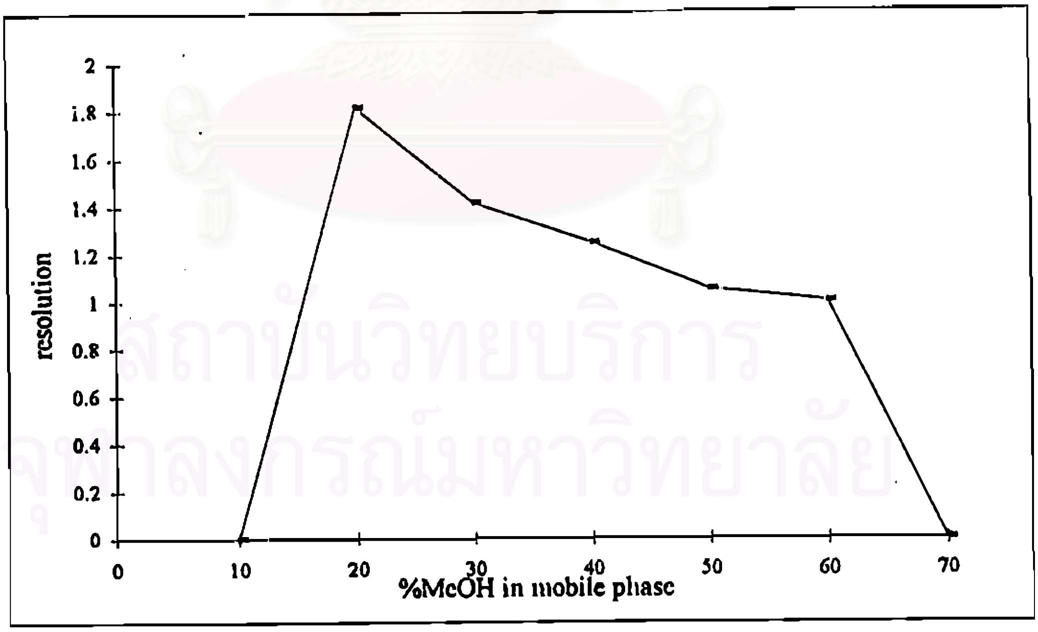

Figure 4.2.6: The relationship between the resolution of inorganic and methylmercury and percent methanol in mobile phase in sodium hexanesulfonate-2-mercaptoethanol system.

The effects of methanol composition in mobile phase of sodium hexanesulfonate system are compared with the 2-mercaptoethanol system. The same percent methanol is used. The mixtures methanol-water buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 5.00 containing only 0.0040% v/v 2-mercaptoethanol are used as the mobile phase for the comparison system. The results of effects of methanol composition in mobile phase are presented in Table 4.2.5. The comparisons between two system are shown in Figure 4.2.7-4.2.9

Table 4.2.5: The results of the effect of the methanol composition in mobile phase on the chromatographic parameters for the separation of mercury compounds in 2-mercaptoethanol system. (Triplicate analyses)

%MeOH	compound	t_R (min)	k'	α*	R_s**
10	solvent	3.058±0.004	-		
	$MeHg^+$	10.495±0.049	2.432	1.032	0.508
	Hg^{2+}	10.731±0.011	2.509		
20	solvent	2.941±0.028	-		
	Hg^{2+}	7.175±0.042	1.440	1.00	0.000
	$MeHg^+$				
30	solvent	2.878±0.015	-		
	Hg^{2+}	4.872±0.004	0.693	1.338	1.854
	$MeHg^+$	5.545±0.019	0.927		
	$PhHg^+$	27.628±0.256	8.600		
40	solvent	2.845±0.018	-		
	Hg^{2+}	4.089±0.004	0.437	1.441	1.175
	$MeHg^+$	4.612±0.008	0.621		
	$PhHg^+$	14.709±0.105	4.170		
50	solvent	2.886±0.041	-		
	Hg^{2+}	3.636±0.013	0.260	1.519	1.102
	$MeHg^+$	4.026±0.008	0.395		
	$PhHg^+$	8.569±0.055	1.969		
60	solvent	2.910±0.023	-		
	Hg^{2+}	3.430±0.026	0.179	1.447	0.829
	$MeHg^+$	3.663±0.004	0.259		
	$PhHg^+$	5.773±0.010	0.934		
70	solvent	2.846±0.038	-		
	Hg^{2+}	3.221±0.003	0.132	1.538	0.774
	$MeHg^+$	3.423±0.005	0.203		
	$PhHg^+$	4.439±0.007	0.560		

* selectivity factor between inorganic and methylmercury

** peak resolution between inorganic and methylmercury

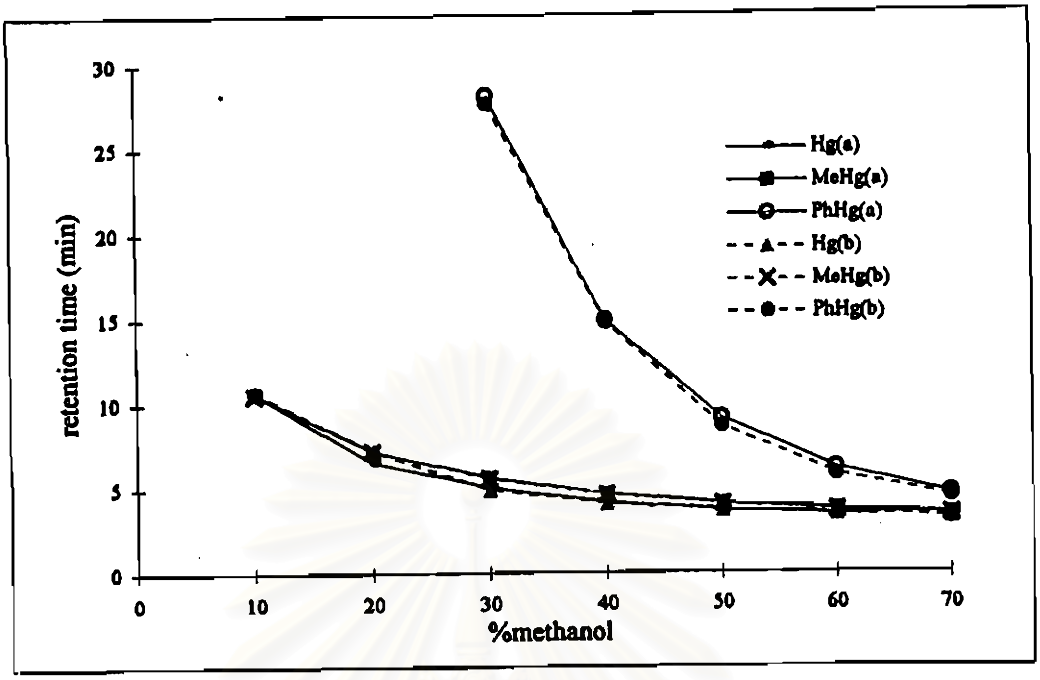

Figure 4.2.7: The relationship between retention time of mercury compounds with percent methanol in (a) sodium hexanesulfonate-2-mercaptoethanol system and (b) 2-mercaptoethanol system

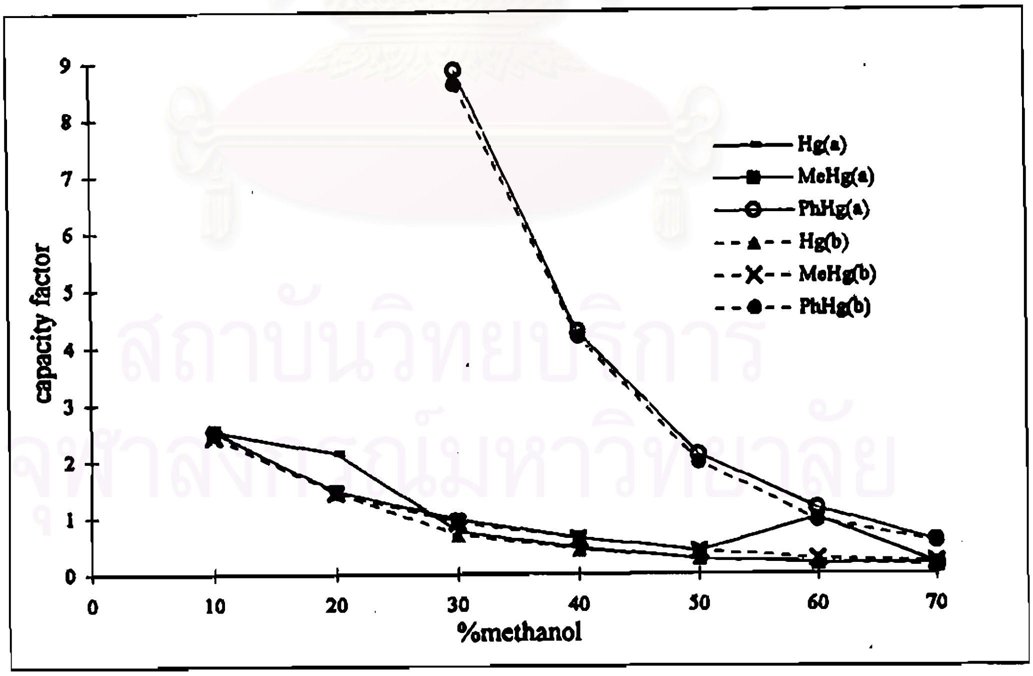

Figure 4.2.8: The relationship between capacity factor of mercury compounds with percent methanol in (a) sodium hexanesulfonate -2-mercaptoethanol system and (b) 2-mercaptoethanol system

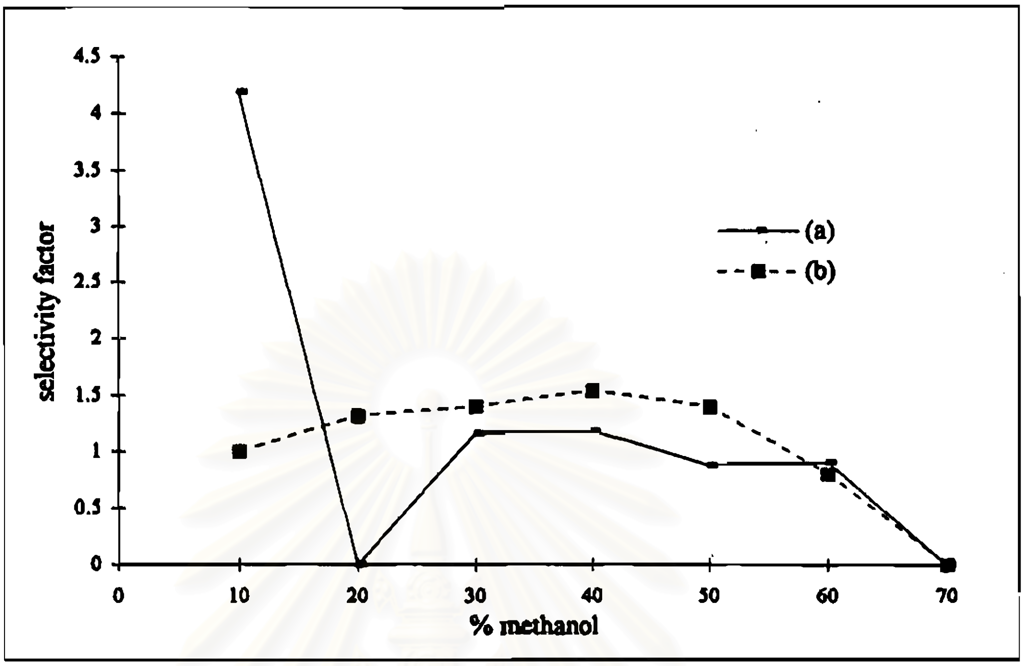

Figure 4.2.9: The relationship between selectivity factor of mercury compounds with percent methanol in (a) sodium hexanesulfonate -2-mercaptoethanol system and (b) 2-mercaptoethanol system

As in the tetrabutylammoniumbromide-2-mercaptoethanol system, the sodium hexanesulfonate-2-mercaptoethanol system has 2 retention mechanisms. The competition between ion-pair formation of mercury compounds and hexanesulfonate and the mercaptoethanol complexes formation are occurred. Considering the stability constant for both formations. The stability constants for mercaptoethanol complexes are 10^6-10^8, whereas ion-pair formations are 10^8. It is show that the complexes formation is the dominated-mechanism. In mobile phase containing ion-pairing agent, sodium hexanesulfanate, the hexanesulfonate is partitioned into the C_{18} stationary phase. In contrast, this system the only dynamic cation-exchange is shown. In low proportion of methanol, the mercaptoethanol complexes are ionised as the previous mobile phase system. The ion-pair mechanism is dominated. However, the ionised groups of inorganic mercury complex is repulsed from the negatively charged hexanesulfonate. The inorganic mercury is less retained than in the conventional C_{18} surface. Thus, the decrease in retention times of inorganic mercury are found in the sodium hexanesulfonate-2-mercaptoethanol system compared with the 2-mercaptoethanol system. When the proportion of methanol in mobile phase is increased, the partition of mercury-complexes is the main mechanism. Non-polar property of stationary phase is not increased with hexanesulfonate, so the retention times of mercury compounds are not significantly changed.

4.1.1.6 The Effect of Mobile Phase Flow Rate

The effects of mobile phase flow rate on the retention time, peak resolution, and peak shape of mercury compounds at the optimum mobile phase conditions are studied. The mixture of methanol-water (40:60% v/v) buffered with 1.00 x 10^{-3}M acetate-acetic acid buffer pH 5.00 containing 0.0040M sodium hexanesulfonate and 0.0040% v/v 2-mercaptoethanol is served as the optimum mobile phase composition. The mobile phase flow rates are varied from 0.80 to 1.20 mL/min in order to find the optimum flow rate of sodium hexanesulfonate-2-mercaptoethanol system according to experimental section 3.5.1.6 and Table 3.2.6. The effects of mobile phase flow rate are presented in Table 4.2.5 and Figure 4.2.7. From the study, the slow flow rates, tailing peaks of inorganic and phenylmercury are found. When flow rates are increased, the peaks of mercury compounds are eluted quickly, the tailing of peak is decreased, and the separation between inorganic and methylmercury is decreased. In lower flow rate, the higher resolution is found. However, long retention time of phenylmercury and broadness peak shape are found in low flow rate. For the appropriate detection of all mercury compounds and the pressure of HPLC system, the flow rate 1.20 mL/min is chosen for the optimum mobile phase flow rate and used for the next study.

Table 4.2.6: The results of the effect of mobile phase flow rate on the chromatographic parameters for the separation of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system. (replicate analyses, n=5)

flow rate (mL/min)	compound	t_R (min)	W (min)	R_s
0.80	solvent	3.509±0.010	-	1.517
	Hg^{2+}	5.058±0.008	0.429±0.076	
	$MeHg^+$	5.689±0.005	0.403±0.113	
	$PhHg^+$	18.119±0.081	0.823±0.064	
1.00	solvent	2.876±0.003	-	1.222
	Hg^{2+}	4.077±0.003	0.397±0.057	
	$MeHg^+$	4.577±0.012	0.422±0.045	
	$PhHg^+$	14.380±0.124	0.880±0.080	
1.20	solvent	2.394±0.005	-	1.072
	Hg^{2+}	3.408±0.006	0.384±0.057	
	$MeHg^+$	3.824±0.009	0.393±0.015	
	$PhHg^+$	11.801±0.045	0.770±0.038	
1.50	solvent	1.959±0.030	-	1.099
	Hg^{2+}	2.746±0.008	0.317±0.043	
	$MeHg^+$	3.089±0.010	0.308±0.022	
	$PhHg^+$	9.389±0.073	0.693±0.085	

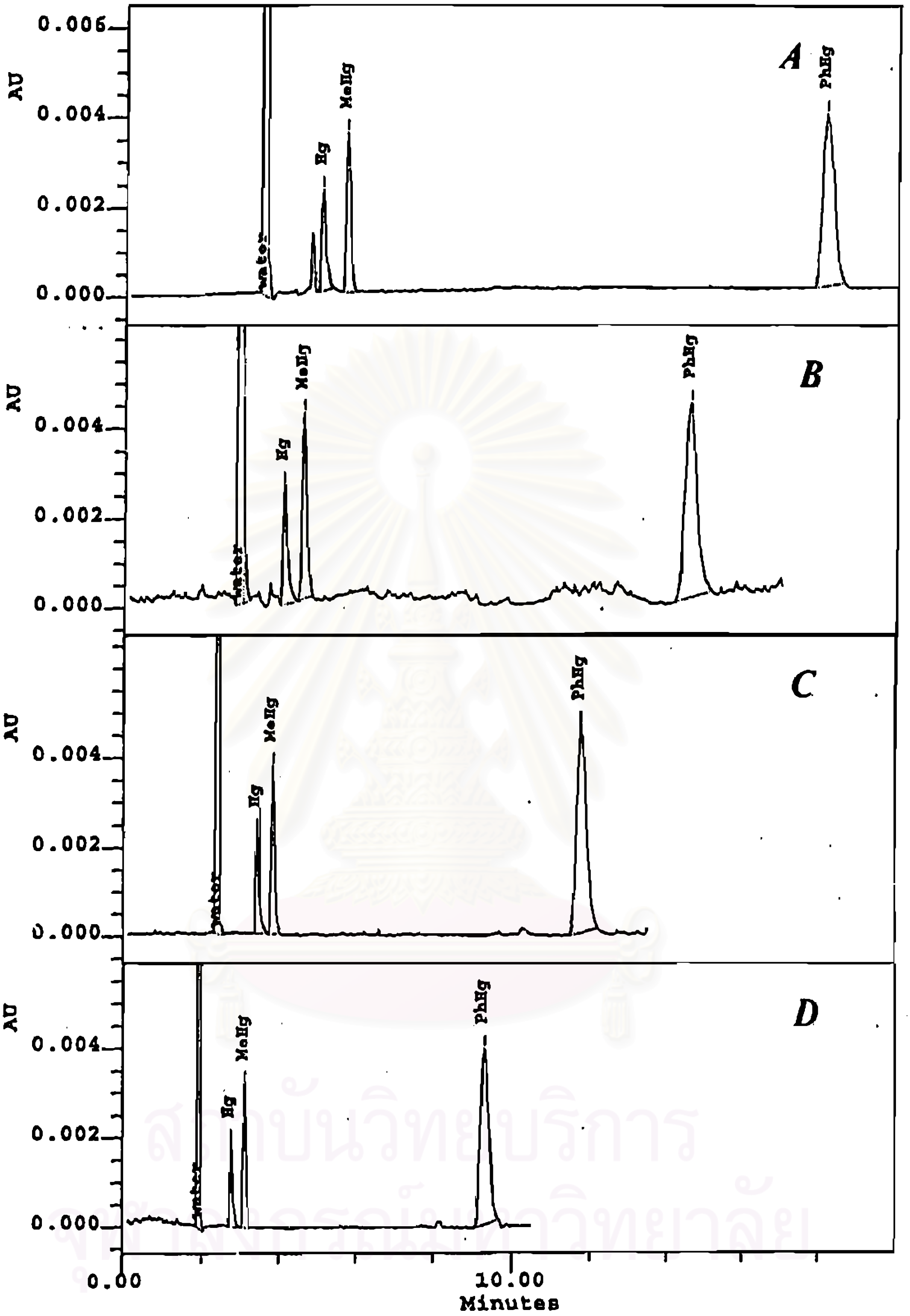

Figure 4.2.10: The chromatograms of mercury compounds at variety of mobile phase flow rate in sodium hexanesulfonate-2-mercaptoethanol system.(chromatographic conditions are shown in Table 3.2.6)

A) 0.80 mL/min B) 1.00 mL/min

C) 1.20 mL/min D) 1.50 mL/min

4.2.2 The Study of the Injection Volume on the Response Signals of Mercury Compounds

The injection volume of mercury compounds is affected to the peak height and peak area. The effects of injection volume on the response signals of mercury compounds are studied under optimum mobile phase conditions from the previous studies according to experimental section 3.5.2 and Table 3.2.7. The results are presented in Table 4.2.6 and Figures 4.2.8-4.2.10. It is found that the highest peak height signal and peak area signal per nanogram of mercury compounds are obtained in volume 10 μL and the highest peak area signal is obtained in volume 10 μL. The injection volume 50 μL signal responses of mercury compounds are lower than in 10 μL, but higher than in other injection volumes. However, the injection volume 50 μL can gives the same amount of mercury compounds with the lower concentration than in the injection volume 10 μL. For the compomised for response signal and the preparation of the concentrations of standard solution the 50 μL is chosen for the next study.

Table 4.2.7: The results of the effect of injection volume on the response signal of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system. (replicate analyses, n=5)

volume (μL)	compound	Area (μV.sec)	response signal (μV.sec/ng)	Height (μV)	response signal (μV/ng)
10	Hg^{2+}	18648±7549	372.96	1581±357	31.62
	$MeHg^+$	18513±3904	185.13	2111±277	21.11
	$PhHg^+$	46369±7513	463.69	2323±133	23.23
20	Hg^{2+}	22220±3476	222.20	2320±261	23.20
	$MeHg^+$	27595±1689	137.98	3624±78	18.12
	$PhHg^+$	6389±02985	319.45	3360±73	16.80
25	Hg^{2+}	21970±1725	175.77	2894±226	23.15
	$MeHg^+$	2929±3713	117.17	3971±31	15.88
	$PhHg^+$	79469±3145	317.88	4206±228	16.82
50	Hg^{2+}	62647±4899	250.59	7522±430	30.09
	$MeHg^+$	74654±1681	149.31	9095±97	18.19
	$PhHg^+$	181783±2928	363.57	8564±89	17.13
100	Hg^{2+}	50039±5413	100.08	14137±1035	28.27
	$MeHg^+$	126723±1809	126.73	1782±748	17.83
	$PhHg^+$	395317±23603	395.32	18685±1030	18.68

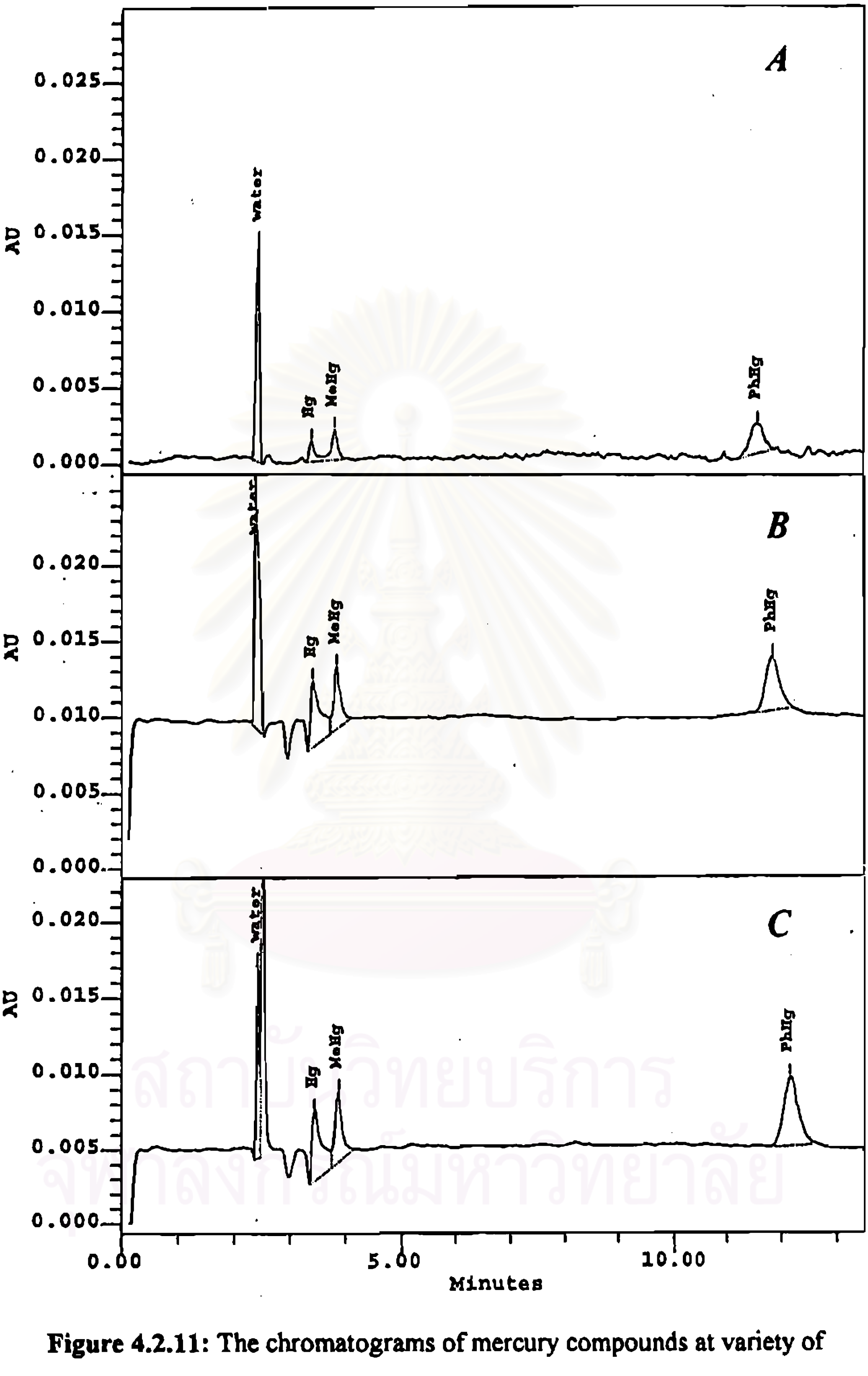

Figure 4.2.11: The chromatograms of mercury compounds at variety of injection volume in sodium hexanesulfonate-2-mercaptoethanol system. (chromatographic conditions are shown in Table 3.2.7)

A) 10 μL B) 20 μL C) 25 μL D) 50 μL E) 100 μL

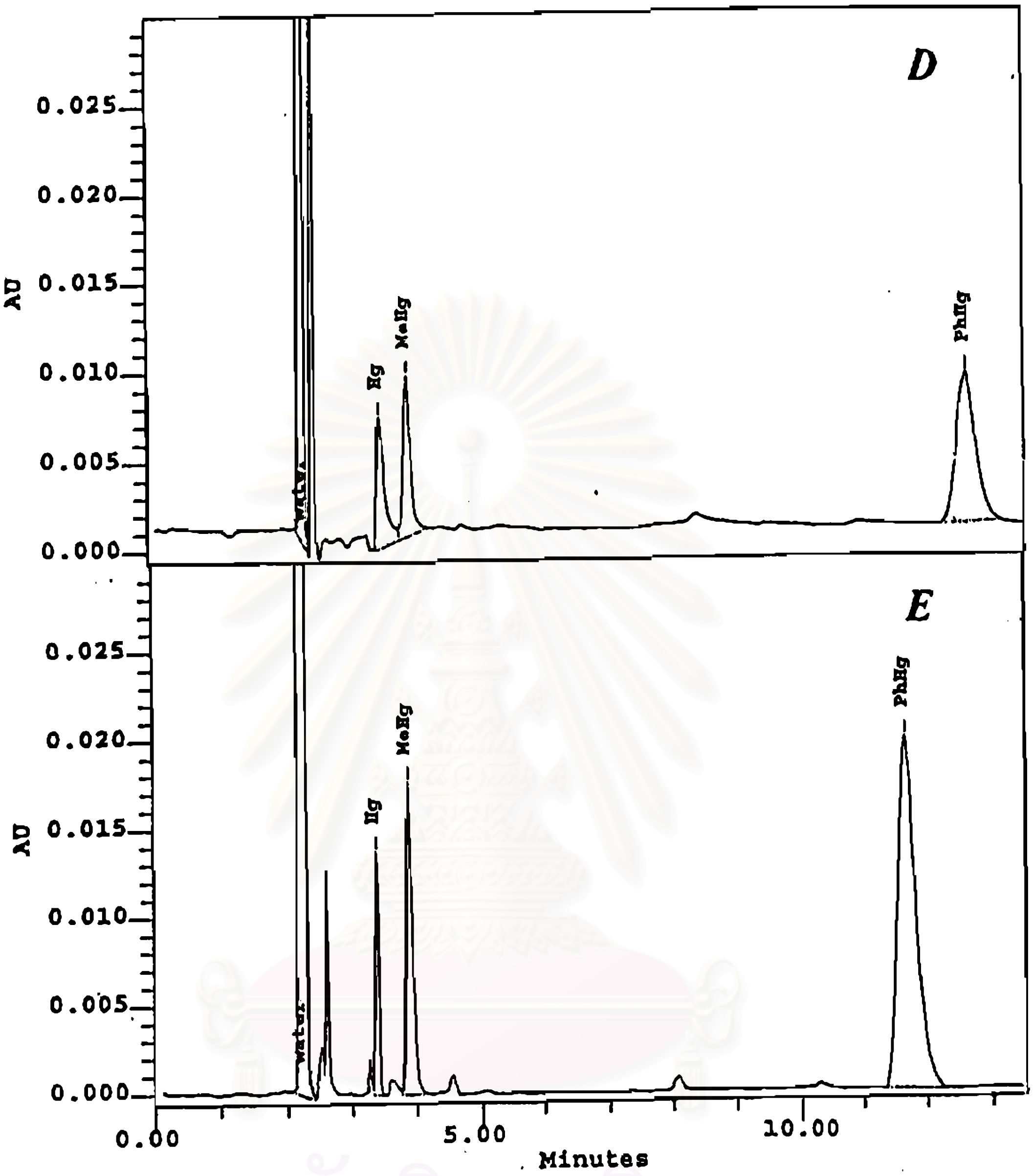

Figure 4.2.11 (continue)

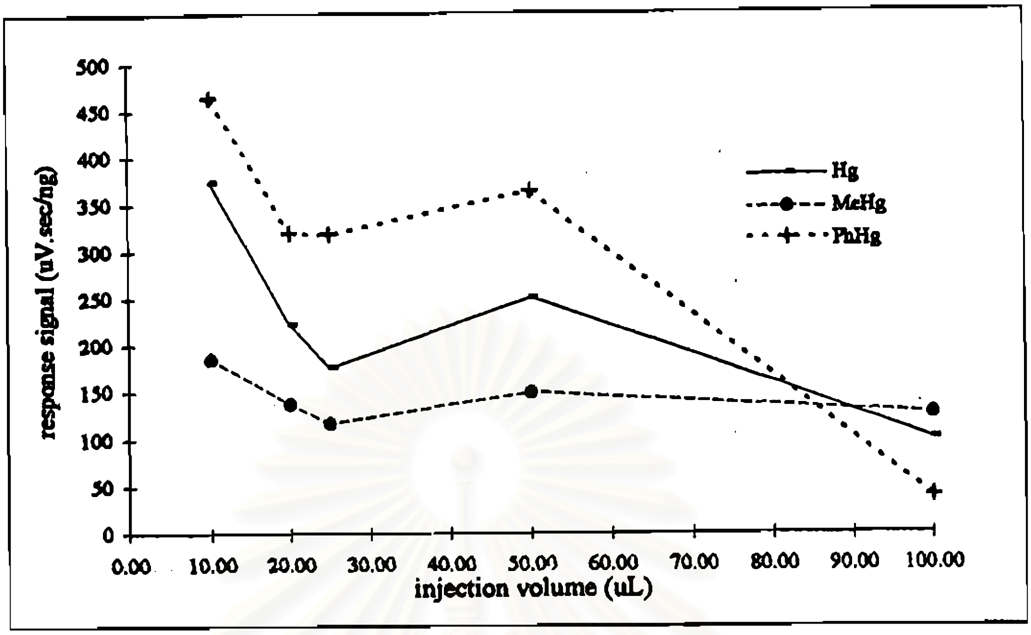

Figure 4.2.12: The relationship between the response signals (peak area) of mercury compounds and the injection volume in sodium hexanesulfonate-2-mercaptoethanol system.

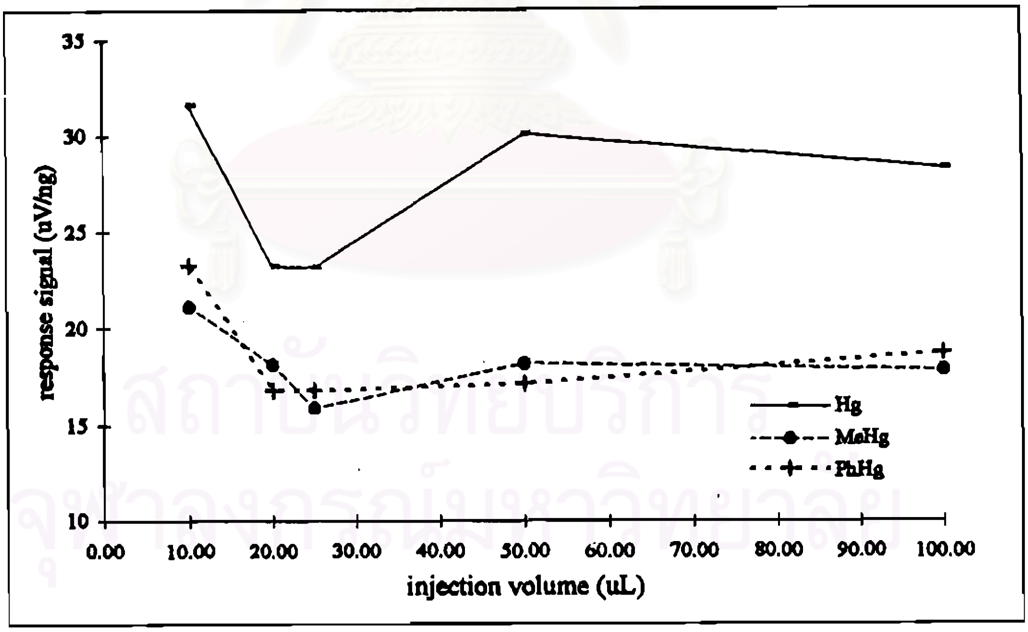

Figure 4.2.13: The relationship between the response signals (peak height) of mercury compounds and the injection volume in sodium hexanesulfonate-2-mercaptoethanol system.

Form the studies of various effects on the resolution and sensitivity of the system the optimum conditions for the next study are shown in the Table 4.2.8.

Table 4.2.8: The optimum conditions of the sodium hexanesulfonate-2-mercaptoethanol system

conditions	parameters
Column	Hypersil, 4.6 x 250 mm., 5μm
Mobile phase composition	40:60% v/v methanol-water buffered with 1.00×10^{-3} M acetate-acetic acid pH 5.00 containing 0.0040M sodium hexanesulfonate and 0.0040% v/v 2-mercaptoethanol
Mobile phase flow rate	1.20 mL/min.
Injection volume	50 μL
Detector	Photodiode Array Detector
Data Acquisition	Maximum plot (200-400 nm)

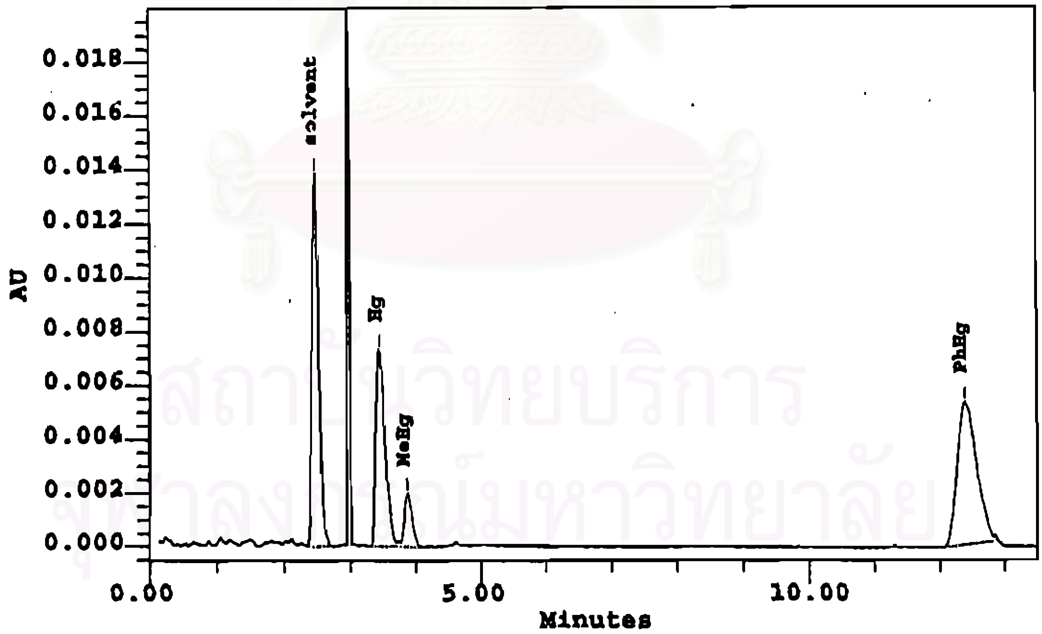

Figure 4.2.14: The chromatogram of mercury compounds under the optimum chromatographic conditions in sodium hexanesulfonate-2-mercaptoethanol system. (chromatographic conditions are shown in Table 4.2.8)

4.2.3 The Study of Linearity of Standard Mercury Compounds

The studies of linearity of standard mercury compounds according to experimental section 3.5.3. In this study, standard mercury compounds are injected under chromatographic conditions in Table 4.2.8. The results are shown in Table 4.2.9. Each peak area and peak height are plotted as the function of the concentration and shown in Figures 4.2.15-4.2.20.

From the Figure 4.2.15, 4.2.17, and 4.2.19, the large linearity and good linear correlation of mercury compounds are observed. Inorganic mercury has linearity over 500.00 ppm. Methylmercury have linearity over 400.00 ppm. and phenylmercury have linearity over 300.00 ppm. From the Figures 4.2.16, 4.2.18, and 4.2.20, the good linearity correlation of mercury componds are also observed. However, in these relationships inorganic mercury has linearity over 100.00 ppm. Methylmercury has linearity over 300.00 ppm. and phenylmercury has linearity over 300.00 ppm. It is found that the shorter linearity range and poorer percent relative deviation and linear coefficient are found in peak height measurement compare with peak area. The only peak area is chosen for the next study. Therfore, the concentration in this range can be used for the study of calibration curve.

Table 4.2.9: The results of the study of linearity of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system..
(replicate analyses, n=5)

Concentration (ppm)	Compound	Peak Area (µV.sec)	%RSD.	Peak Height (µV)	%RSD.
5.00	Hg^{2+}	53068	8.90	4577	8.85
	$MeHg^{+}$	18864	6.20	2321	3.43
	$PhHg^{+}$	52708	5.25	4209	1.35
10.00	Hg^{2+}	133108	7.99	13130	8.84
	$MeHg^{+}$	84072	4.38	6482	4.82
	$PhHg^{+}$	205950	1.33	8626	2.97
20.00	Hg^{2+}	293433	4.17	30002	9.97
	$MeHg^{+}$	146381	2.91	11998	5.52
	$PhHg^{+}$	411617	1.81	17339	2.46
50.00	Hg^{2+}	693841	0.92	67257	4.80
	$MeHg^{+}$	161481	2.18	16785	1.27
	$PhHg^{+}$	987981	2.67	41872	1.48
100.00	Hg^{2+}	1284575	1.77	111674	4.76
	$MeHg^{+}$	314758	7.89	27704	3.48
	$PhHg^{+}$	2073907	1.81	77115	4.31
200.00	Hg^{2+}	3001707	3.75	272954	2.71
	$MeHg^{+}$	760738	3.38	69949	3.11
	$PhHg^{+}$	3932487	0.32	124969	2.18
300.00	Hg^{2+}	4441198	0.60	402475	0.52
	$MeHg^{+}$	1069163	1.06	97273	0.69
	$PhHg^{+}$	6221361	1.61	141630	1.83
400.00	Hg^{2+}	5805371	0.44	694327	1.10
	$MeHg^{+}$	1458263	2.72	93567	0.55
	$PhHg^{+}$	-	-	-	-
500.00	Hg^{2+}	7792353	0.27	925806	0.37
	$MeHg^{+}$	-	-	-	-
	$PhHg^{+}$	-	-	-	-

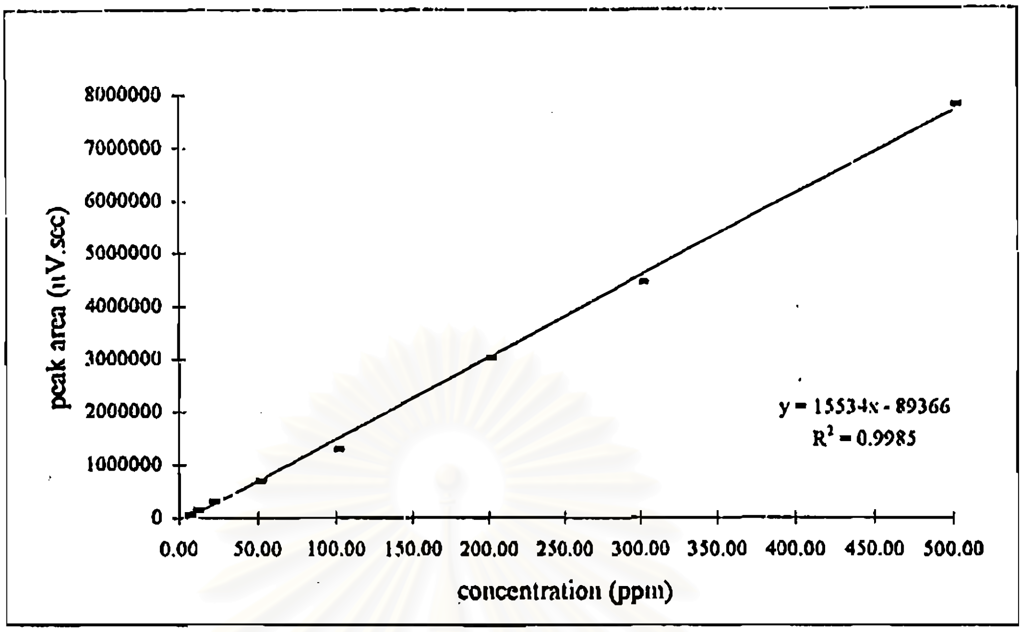

Figure 4.2.15: The relationship between the peak area of inorganic mercury and the concentration in sodium hexanesulfonate-2-mercaptoethanol system.

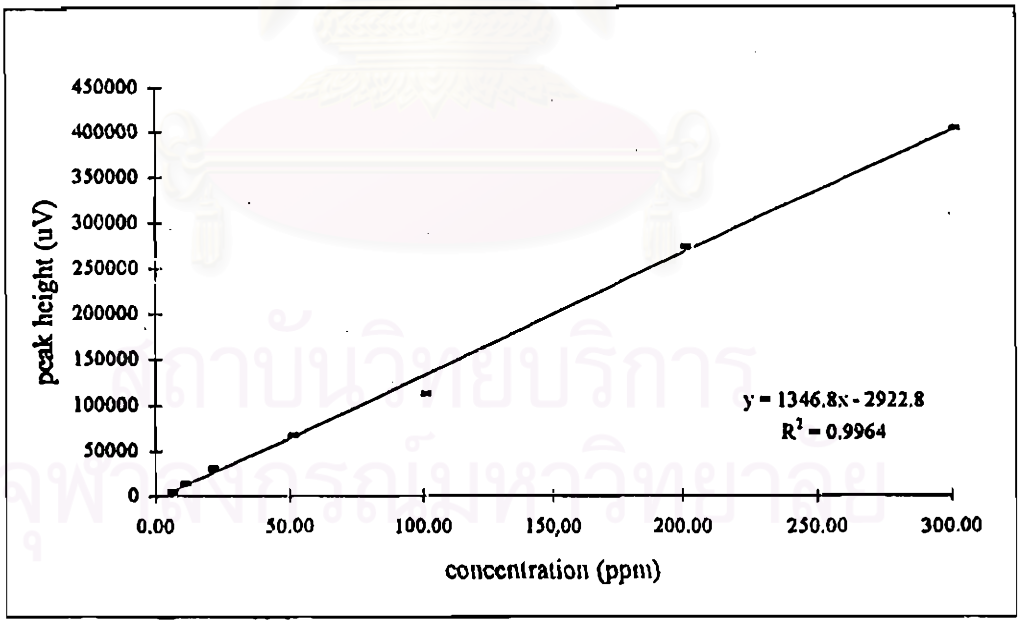

Figure 4.2.16: The relationship between the peak height of inorganic mercury and the concentration in sodium hexanesulfonate-2-mercaptoethanol system.

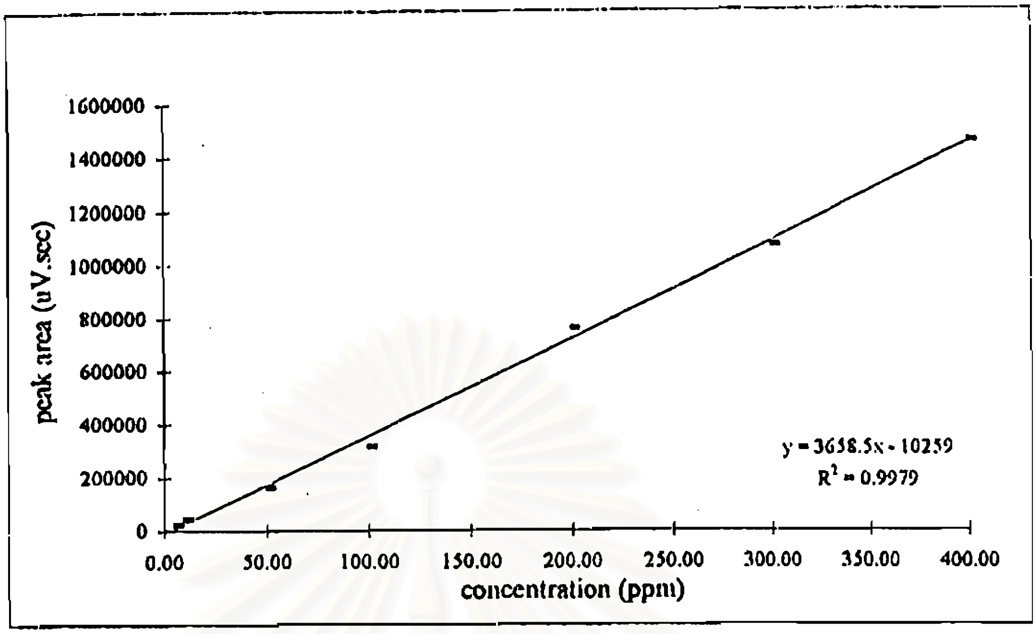

Figure 4.2.17: The relationship between the peak area of methylmercury and the concentration in sodium hexanesulfonate-2-mercaptoethanol system.

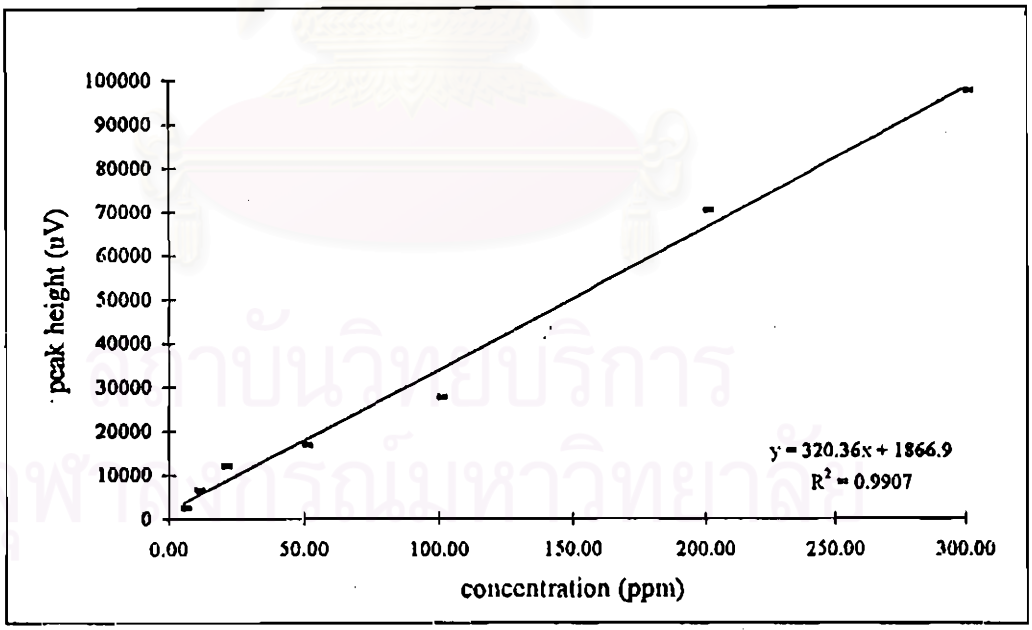

Figure 4.2.18: The relationship between the peak height of methylmercury and the concentration in sodium hexanesulfonate-2-mercaptoethanol system.

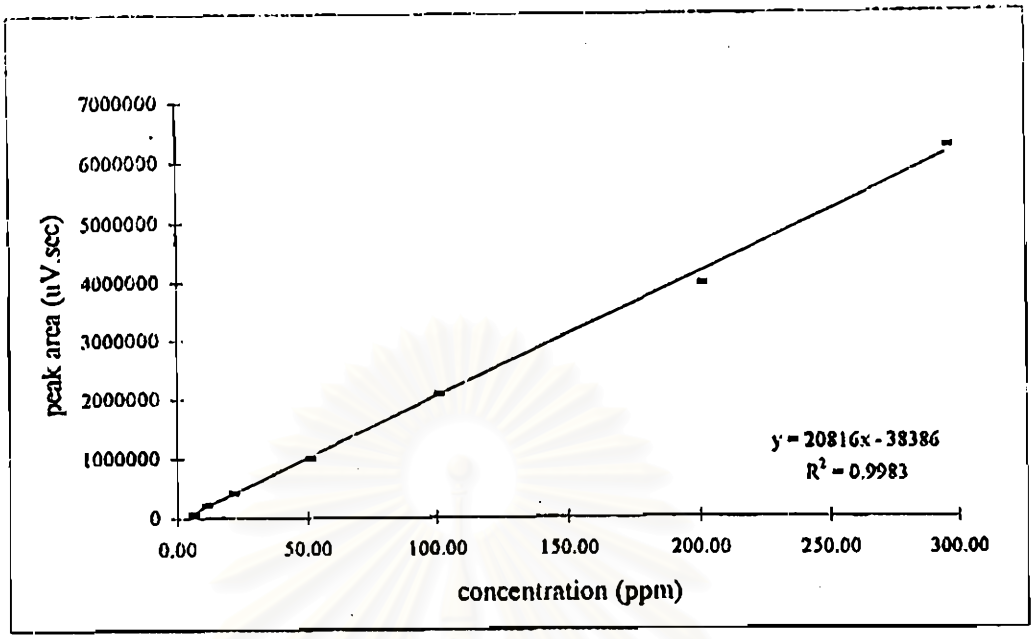

Figure 4.2.19: The relationship between the peak area of phenylmercury and the concentration in sodium hexanesulfonate-2-mercaptoethanol system.

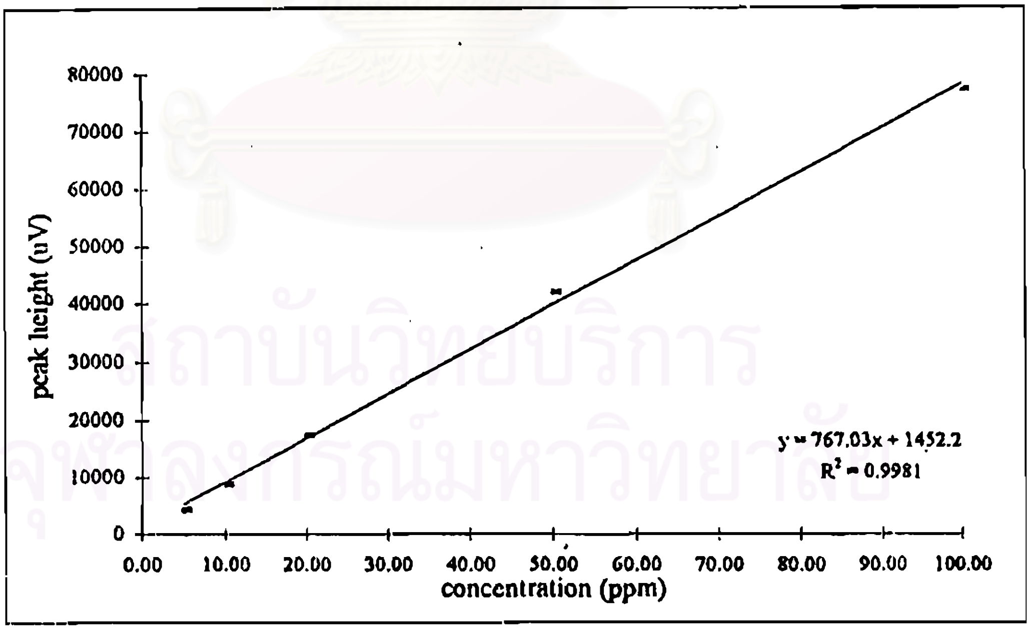

Figure 4.2.20: The relationship between the peak height of phenylmercury and the concentration in sodium hexanesulfonate-2-mercaptoethanol system.

4.2.4 The Study of Calibration Curve of Mercury Compounds

The calibration curve of mercury compounds in the range of 1.00-20.00 ppm were study according to the experimental section 3.5.4. The relationship between the peak area and concentration were shown in Table 4.2.10 and Figures 4.2.21-4.2.23. From the calibration curves show that all of mercury compounds give good linear correlation, R^2 = 0.9958, 0.9996, and 0.9985 for inorganic, methyl, and phenylmercury, respectively. The expression for inorganic mercury is y = 14192x - 17219, methylmercury is y = 6592.7x - 2455.1, and phenylmercury is y = 16895x - 305.5. It is found that phenylmercury and inorganic mercury give higher sensitivity than methylmercury.

Table 4.2.10: The results of the study of calibration curve of mercury compounds in sodium hexanesulfonate-2-mercaptoethanol system.. (replicate analyses, n=5)

Concentration (ppm)	Compound	Peak Area (µV.sec)	%RSD.
1.00	Hg^{2+}	5675	4.23
	$MeHg^{+}$	4914	16.00
	$PhHg^{+}$	10042	6.79
3.00	Hg^{2+}	18233	8.17
	$MeHg^{+}$	17962	3.66
	$PhHg^{+}$	57689	6.65
6.00	Hg^{2+}	70415	5.98
	$MeHg^{+}$	36094	2.97
	$PhHg^{+}$	101431	8.48
10.00	Hg^{2+}	119367	8.10
	$MeHg^{+}$	62682	3.00
	$PhHg^{+}$	170114	4.85
15.00	Hg^{2+}	190576	4.09
	$MeHg^{+}$	95711	1.89
	$PhHg^{+}$	24915	2.41
20.00	Hg^{2+}	273015	3.69
	$MeHg^{+}$	130504	0.81
	$PhHg^{+}$	338939	5.99

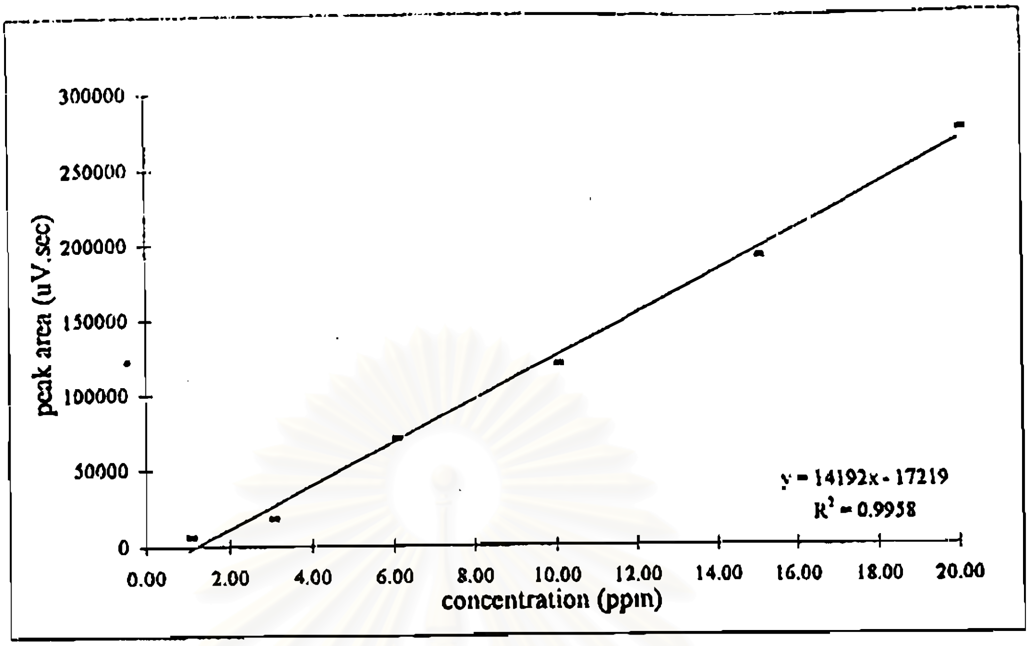

Figure 4.2.21: The calibration curve of inorganic mercury in sodium hexanesulfonate-2-mercaptoethanol system.

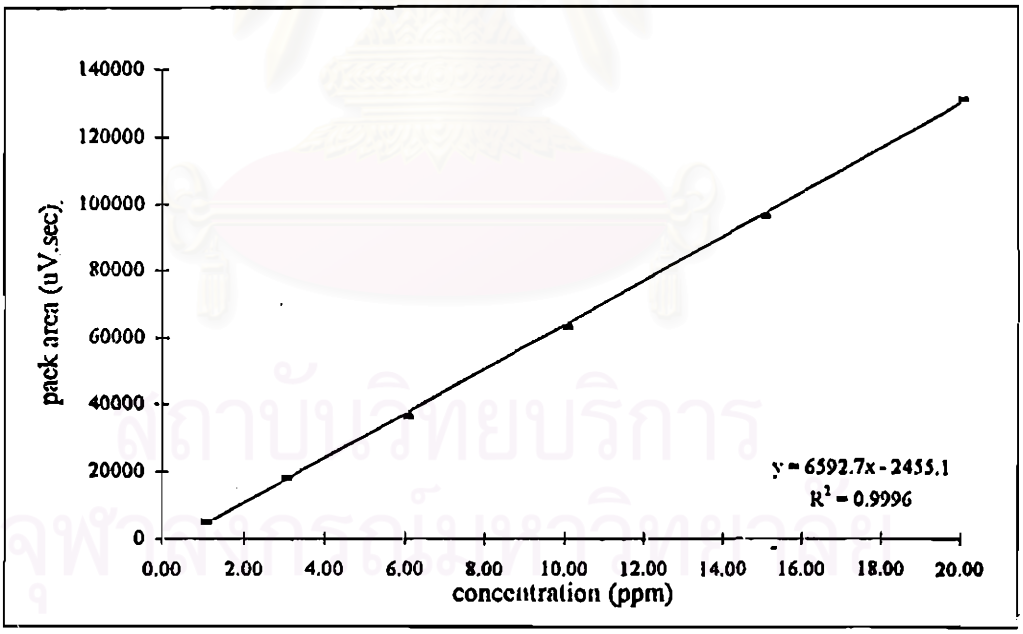

Figure 4.2.22: The calibration curve of methylmercury in sodium hexanesulfonate-2-mercaptoethanol system.

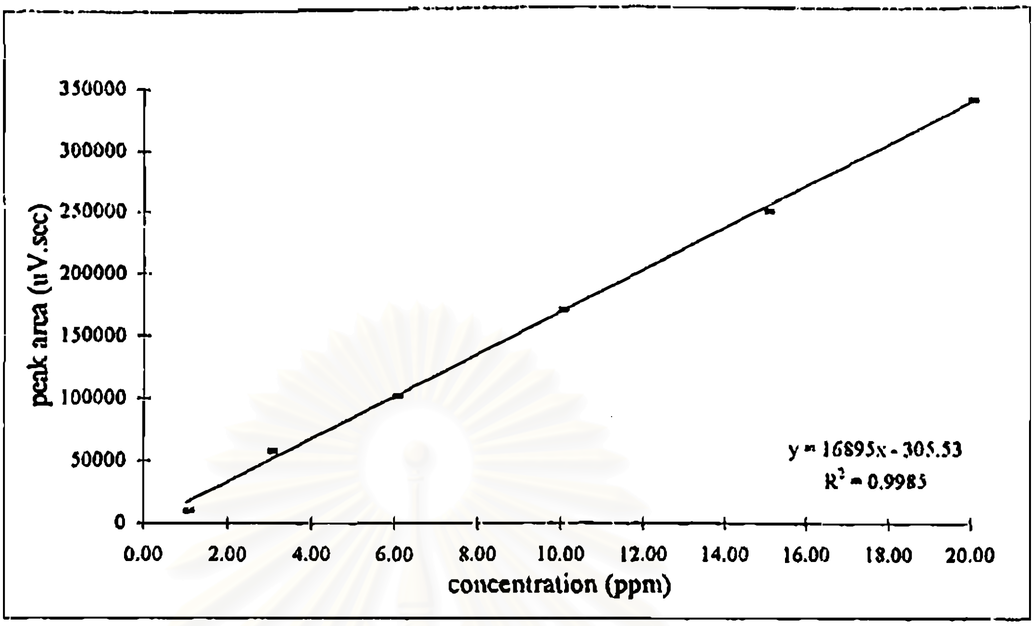

Figure 4.2.23: The calibration curve of phenylmercury in sodium hexanesulfonate-2-mercaptoethanol system.

4.2.5 The Study of Detection Limit of the System

The study of detection limit for sodium hexanesulfonate-2-mercaptoethanol system according to the experimental section 3.5. Inorganic mercury has the high detection limits of 0.80 ppm as Hg (40 ng as Hg). Methylmercury is 0.30 ppm as Hg (15 ng as Hg) and phenylmercury is 0.20 ppm as Hg (10 ng as Hg). Inorganic mercury gives the high detection limit because it eluted first and closed to the solvent peak. The solvent peak in this system is the negative peak, and the low concentration signal of inorganic mercury is effected from the solvent peak. The detection limit of this system will compare with the detection limit of tetrabutylammonium bromide-2-mercaptoethanol system and are presented in Table 4.2.12

4.2.6 The Study of Precision of the System

The study of precision for sodium hexanesulfonate-2-mercaptoethanol system according to the experimental section 3.5.6 and the AOAC® Peer-Verified Methods Program (87) are present in Table 4.2.11. The percent relative standard deviations of all concentration in the calibration curve are less than the acceptability percent relative standard deviation from the AOAC method except in concentration 12.00 ppm of methylmercury but this percent relative standard deviation is closed to the standard method. When compare all of mercury compounds, phenylmercury gives the least deviation in all concentration. In low concentration (4.00-8.00 ppm) mercury compounds give high deviation, but deviation are decreased with the increased concentration. It is shown that the precision of this method is acceptable. The comparison with tetrabutylammonium bromide-2-mercaptoethanol are present in Table 4.2.12

Table 4.2.11: The result of the study of precision for the sodium hexane sulfonate-2-mercaptoethanol system compare with the acceptable percent relative standard deviation of the reference method

concentration (ppm)	Hg^{2+}		$MeHg^{+}$		$PhHg^{+}$		AOAC
	peak area (µV.sec)	%RSD	peak area (µV.sec)	%RSD	peak area (µV.sec)	%RSD	%RSD
4.00	67711	7.12	32615	5.11	58040	5.50	8.71
	71132		30802		62242		
	66358		29597		57473		
	60129		33314		53534		
	68837		30249		59739		
8.00	104695	5.73	87778	6.89	146739	6.00	7.85
	114550		90554		135146		
	110447		94190		151255		
	98255		104873		139222		
	107487		93742		156744		
12.00	156218	2.00	114989	7.46	282507	3.24	7.37
	158228		116472		268562		
	156119		137723		263778		
	150793		128387		262756		
	158633		124623		278046		
16.00	201168	6.72	159394	4.94	367307	4.94	7.07
	184219		163758		334145		
	179070		147708		361813		
	178585		163884		366685		
	205564		168580		383246		

Table 4.2.12: The comparison of analytical parameters from tetrabutylammonium bromide-2-mercaptoethanol system and sodium hexanesulfonate-2-mercaptoethanol system under optimum condition for each system.

Analytical parameters	Tetrabutylammonium bromide-2-mercaptoethanol system		Sodium hexanesulfonate-2-mercaptoethanol system		
	Hg^{2+}	$MeHg^+$	Hg^{2+}	$MeHg^+$	$PhHg^+$
retention time(min)	6.898±0.097	6.098±0.038	3.408±0.006	3.824±0.009	11.801±0.045
linearity	$y=7168.3x+9180.9$ $R^2 = 0.9983$	$y = 5836.9x-1771.1$ $R^2 = 0.9998$	$y = 15534x-89366$ $R^2 = 0.9985$	$y = 3658.5x-10259$ $R^2 = 0.9979$	$y = 20816x-38386$ $R^2 = 0.9983$
calibration curve	$y = 9363.6x-4750.3$ $R^2 = 0.9995$	$y = 6333.7x-3623.7$ $R^2 = 0.9976$	$y = 14192x-17219$ $R^2 = 0.9958$	$y = 6592.7x-2455.1$ $R^2 = 0.9996$	$y = 16895x-305.5$ $R^2 = 0.9985$
detection limit	0.30 ppm (as Hg) 15 ng (as Hg)	0.30 ppm (as Hg) 15 ng (as Hg)	0.80 ppm (as Hg) 40 ng (as Hg)	0.30 ppm (as Hg) 15ng (as Hg)	0.20ppm (as Hg) 10 ng (as Hg)
precision*	7.04%, 1.40%, 2.00%, and 4.10%	6.25%, 2.74%, 3.28%, and 2.38%	7.12%, 5.73%, 2.00%, and 6.72%	5.11%, 6.89%, 7.46%, and 4.94%	5.50%, 6.00%, 3.24%, and 4.94%

* percent relative standard deviation for 4.00, 8.00, 12.00, and 16.00 ppm, respectively.

From Table 4.2.12, various analytical parameters of two mobile phase systems are compared. In tetrabutylammonium bromide-2-mercaptoethanol system (first system), inorganic and methylmercury are completely separated within 8 min, but phenylmercury is not detected. The phenylmercury is detected in the sodium hexanesulfonate system (second system) with higher sensitivity and detection limit over other compounds. In first system inorganic mercury is eluted after methylmercury and complete separation, but in second system inorganic mercury is eluted before methylmercury. It is show that the first system is suitable for the speciation of inorganic and methylmercury, especially methylmercury, in environmental. In the environmental, the concentration of methylmercury is very less than inorganic mercury. In the system that inorganic eluted first, the high concentration of inorganic mercury is affected to the peak of methylmercury. From the elution order of the first system, the problem can be resolved. On the other hand, the determination of all inorganic and organomercury compounds the second system is suitable than the first one.

In addition, the detection limit and sensitivity of two system are compared. The detection limit of inorganic mercury in the second system is very poor resulted from inorganic peak is closed to the solvent peak as discussed in the section 4.2.5. For methylmercury the detection limit and sensitivity are similarity in two systems. Also in second system, inorganic mercury has the same detection limit as methylmercury. However, the sensitivity of inorganic mercury is higher than methylmercury about two time. This indicates that the mercury-mercaptoethanol complexes are affected the responses of detector. The sensitivity is dependent on the chromophore group in the molecule and the type of complexes. The ion-pairing agent is not affected. As the results show that the ion-pair agent is affected to the only separation mechanism. The comparison of the study of precision of the system. Both system have the acceptable %RSD compared with the AOAC® reference method.