

## **CHAPTER 3**

### **RESULTS**

#### **3.1 Optimization of Ion Chromatographic Conditions**

The ion chromatographic conditions were optimized for study the separation of chlorine-containing anions by using three anion-exchange columns, which were an IonPac AS4A column, an IonPac AS4A-SC column and a Metrosep A Supp 4 column. A mixed standard solution consisted of 5.0, 1.0, 5.0 and 5.0 ng/ $\mu$ l of chlorine-containing anions, chlorite, chloride, chlorate and perchlorate, respectively, was used. A 100  $\mu$ l of this solution was injected into the ion chromatograph under the investigated conditions.

##### **3.1.1 Optimization of conditions for an IonPac AS4A column**

###### **3.1.1.1 The effects of the eluent concentration**

The effects of the eluent concentration were investigated using a mixture of sodium carbonate and sodium bicarbonate at flow rate of 2.00 ml/min. The results of retention time and resolution are shown in Tables 3.1 and 3.2, respectively.

###### **3.1.1.2 The effects of the eluent flow rate**

The effects of the eluent flow rate were investigated using different composition ratios of sodium carbonate and sodium bicarbonate. The results of retention time and resolution are shown in Table 3.3.

**Table 3.1** Effects of eluent concentration on retention time of chlorine-containing anions with an IonPac AS4A column at flow rate of 2.00 ml/min

Concentration of carbonate : bicarbonate (mM)	Average retention time (min)			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
1:0	1.20	1.57	3.24	42.76
1:1	1.17	1.47	2.88	35.42
1:2	1.10	1.37	2.57	31.43
2:1	1.04	1.24	2.26	27.73
2:2	1.05	1.25	2.21	24.69

**Table 3.2** Effects of eluent concentration on resolution of chlorine-containing anions with an IonPac AS4A column at flow rate of 2.00 ml/min

Concentration of carbonate : bicarbonate (mM)	Average resolution		
	$\text{ClO}_2^- - \text{Cl}^-$	$\text{Cl}^- - \text{ClO}_3^-$	$\text{ClO}_3^- - \text{ClO}_4^-$
1:0	2.05	9.67	12.42
1:1	1.72	7.07	12.64
1:2	1.79	5.91	12.34
2:1	1.38	5.83	13.59
2:2	1.00	4.55	13.42

**Table 3.3** Effects of flow rate on retention time and resolution of chlorine-containing anions with an IonPac AS4A column

Chlorine-containing anion	Average retention time (min) using different composition ratio at 3 different flow rate (ml/min)														
	1.00					1.50					2.00				
	2:2	3:3	4:4	5:5		2:2	3:3	4:4	5:5		2:2	3:3	4:4	5:5	
$\text{ClO}_2^-$	2.00	1.93	1.94	2.20		1.40	1.25	1.27	1.46		1.05	0.98	0.95	0.99	
$\text{Cl}^-$	2.50	2.33	2.26	3.53		1.66	1.57	1.48	2.31		1.25	1.15	1.08	1.74	
$\text{ClO}_3^-$	4.41	4.00	3.72	4.42		3.00	2.65	2.42	2.94		2.21	2.01	1.83	2.22	
$\text{ClO}_4^-$	51.93	47.40	40.34	38.20		33.74	30.52	26.32	24.40		24.69	22.50	19.59	17.76	
Anion pair	Average resolution using different composition ratio at 3 different flow rate (ml/min)														
	1.00					1.50					2.00				
	2:2	3:3	4:4	5:5		2:2	3:3	4:4	5:5		2:2	3:3	4:4	5:5	
$\text{ClO}_2^- - \text{Cl}^-$	1.65	1.37	1.23	1.10		1.13	1.09	1.05	0.87		1.00	0.42	0.38	-	
$\text{Cl}^- - \text{ClO}_3^-$	7.14	6.77	6.13	6.09		5.46	4.77	4.23	4.23		4.55	3.26	1.89	1.92	
$\text{ClO}_3^- - \text{ClO}_4^-$	22.35	15.27	14.35	13.58		14.23	13.97	13.81	13.23		13.42	13.29	12.72	12.48	

[ ] : coelution of chlorite and chloride

### 3.1.2 Optimization of conditions for an IonPac AS4A-SC column

#### 3.1.2.1 The effects of the eluent type in isocratic system

The effects of the eluent type were investigated at a flow rate of 2.00 ml/min. The results of retention time, peak area and resolution are shown in Tables 3.4-3.6, respectively.

**Table 3.4** Effects of eluent type on retention time of chlorine-containing anions with an IonPac AS4A-SC at flow rate of 2.00 ml/min

Eluent type	Average retention time (min)			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
10 mM sodium hydroxide	1.42	1.83	3.70	52.17
3 mM sodium benzoate	1.95	2.56	5.19	77.15
0.5 mM sodium oxalate	1.20	1.47	2.65	28.75
1 mM phthalic acid	2.07	2.87	5.17	60.13
1 mM KHP	1.35	1.70	2.90	30.44
12.8 mM sodium carbonate	-	-	1.45	11.34
1 mM sodium citrate	-	-	1.18	8.35
1 mM potassium chromate	-	-	1.63	15.87
acetonitrile	NF	NF	NF	NF

[-] : coelution of chlorite and chloride

NF : not found

**Table 3.5** Effects of eluent type on resolution of chlorine-containing anions with an IonPac AS4A-SC at flow rate of 2.00 ml/min

Eluent type	Average resolution		
	$\text{ClO}_2^- - \text{Cl}^-$	$\text{Cl}^- - \text{ClO}_3^-$	$\text{ClO}_3^- - \text{ClO}_4^-$
10 mM sodium hydroxide	2.55	7.19	16.59
3 mM sodium benzoate	3.67	9.09	26.83
0.5 mM sodium oxalate	1.65	6.11	21.51
1 mM phthalic acid	3.46	7.98	21.94
1 mM KHP	2.05	6.03	21.74
12.8 mM sodium carbonate	-	2.22	9.52
1 mM sodium citrate	-	1.69	13.66
1 mM potassium chromate	-	3.14	19.11
acetonitrile	NF	NF	NF

[-] : coelution of chlorite and chloride

NF : not found

**Table 3.6** Effects of eluent type on peak area of chlorine-containing anions with an IonPac AS4A-SC at flow rate of 2.00 ml/min

Eluent type	Average peak area ( $\times 10^5$ ) (arbitrary unit)			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
10 mM sodium hydroxide	2.21	2.09	2.93	1.81
3 mM sodium benzoate	3.04	2.43	3.45	2.29
0.5 mM sodium oxalate	2.86	2.16	3.72	2.26
1 mM phthalic acid	2.03	1.71	2.67	2.45
1 mM KHP	2.45	1.92	3.28	3.10
12.8 mM sodium carbonate	-	-	3.19	2.21
1 mM sodium citrate	-	-	3.57	2.64
1 mM potassium chromate	-	-	3.79	2.73
acetonitrile	NF	NF	NF	NF

[-] : coelution of chlorite and chloride

NF : not found

### 3.1.2.2 The effects of the eluent type in gradient system

A pair of the eluent types with a equal conductivity value but a different eluent strength were used with an IonPac AS4A-SC column at a flow rate of 2.00 ml/min. The gradient eluent methods were investigated as follow.

(a) method A : 10.0 mM sodium hydroxide [1] and 12.6 mM sodium carbonate[2]

Condition : time 1.4 – 1.5 min %[1] 100 to %[2] 100

(b) method B : 3.0 mM sodium bicarbonate [1] and 12.8 mM sodium carbonate[2]

Condition : time 2.5 – 2.8 min %[1] 100 to %[2] 100

(c) method C : 3.0 mM sodium benzoate [1] and 0.59 mM sodium citrate[2]

Condition : time 1.8 – 2.0 min %[1] 100 to %[2] 100

(d) method D : 1.0 mM sodium benzoate [1] and 0.28 mM sodium citrate[2]

Condition : time 2.0 – 2.5 min %[1] 100 to %[2] 100

(e) method E : 1.0 mM citric acid [1] and 1.0 mM sodium citrate[2]

Condition : time 7.0 – 8.0 min %[1] 100 to %[2] 100

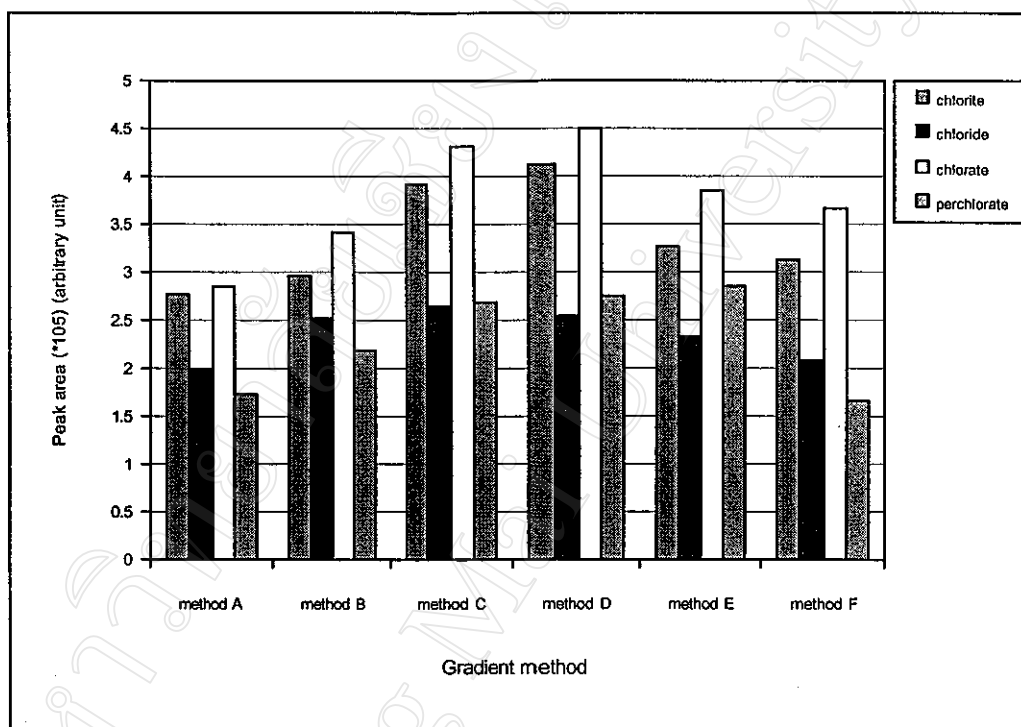
(f) method F : 0.2 mM sodium benzoate [1] and 12.8 mM sodium carbonate[2]

Condition : time 4.5 – 5.0 min %[1] 100 to %[2] 100

The results of retention time and resolution of each anion pair are shown in Table 3.7 and peak area are shown in Figure 3.1. The chromatograms of chlorine-containing anions in each gradient system are presented in Figures 3.2-3.7.

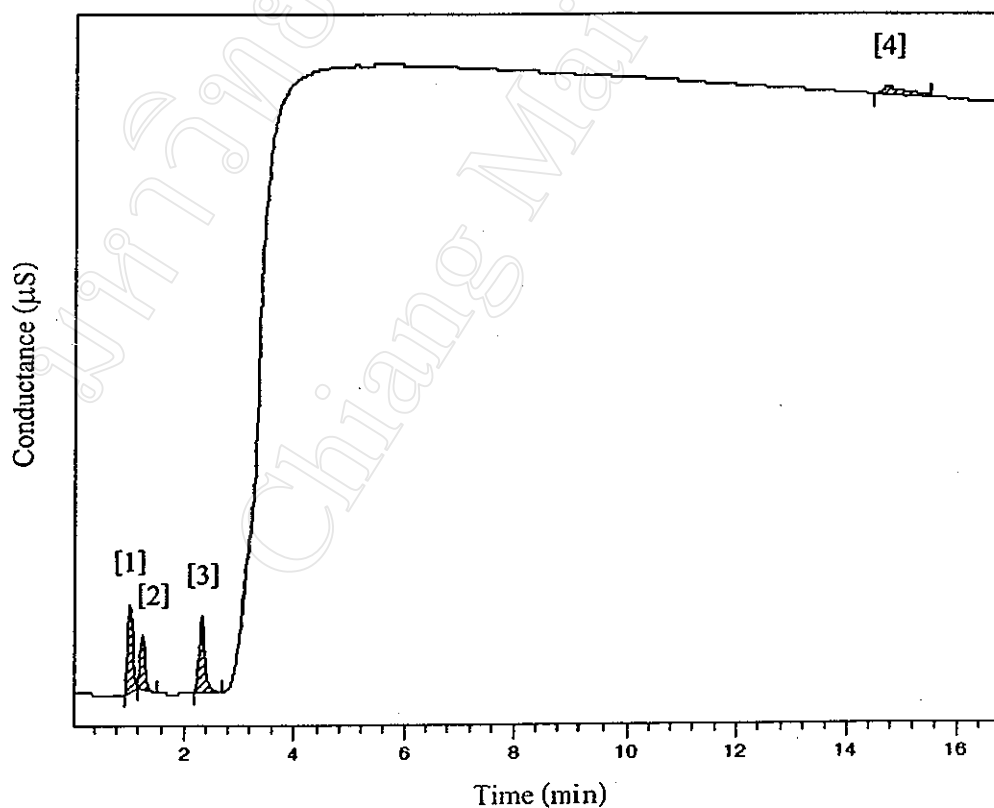
**Table 3.7** Effects of the gradient method on retention time and resolution of chlorine- containing anions with an IonPac AS4A-SC column at flow rate of 2.00 ml/min

Chlorine- containing anion	Average retention time (min) using the gradient method					
	Method A	Method B	Method C	Method D	Method E	Method F
$\text{ClO}_2^-$	1.05	1.13	1.12	1.15	2.29	1.57
$\text{Cl}^-$	1.27	1.40	1.37	1.42	3.23	2.12
$\text{ClO}_3^-$	2.33	2.62	2.43	2.59	6.66	4.60
$\text{ClO}_4^-$	14.77	14.88	12.21	15.63	17.24	17.67
Anion pair	Average resolution using the gradient method					
	Method A	Method B	Method C	Method D	Method E	Method F
$\text{ClO}_2^- - \text{Cl}^-$	1.37	1.39	1.53	1.66	3.83	3.03
$\text{Cl}^- - \text{ClO}_3^-$	6.00	5.44	5.41	5.91	8.90	9.54
$\text{ClO}_3^- - \text{ClO}_4^-$	21.13	15.58	17.47	15.78	16.33	19.23



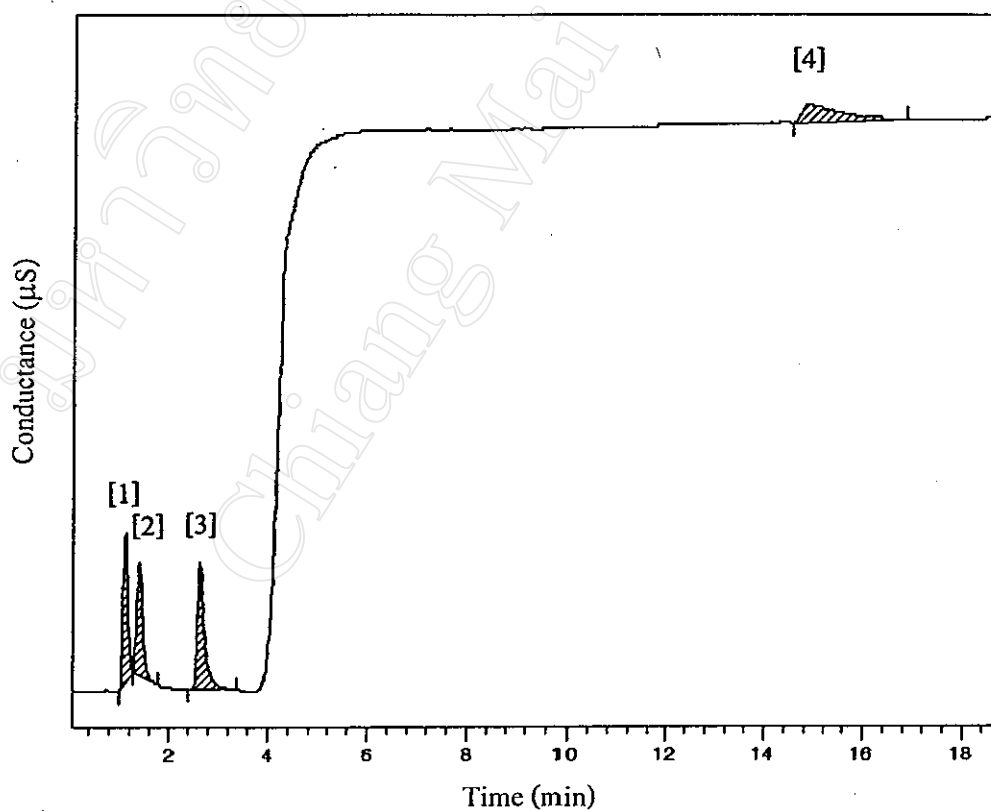
**Figure 3.1** Effects of the gradient method on peak area of chlorine-containing anions with an IonPac AS4A-SC column at flow rate of 2.00 ml/min.

Column	IonPac AS4A-SC (4×250) analytical column IonPac AS4A-SC (4×50) guard column
Eluents	10.0 mM sodium hydroxide [1] and 12.6 mM sodium carbonate[2]
Condition	time 0.0 min %[1] 100 time 1.4 – 1.5 min %[1] 100 to %[2] 100 time 1.5 min %[2] 100
Flow rate	2.00 ml/min
Detector	Conductivity detector, anion self-regenerating suppressor ASRS-1 (4mm) at 100 mA auto-suppression recycle mode



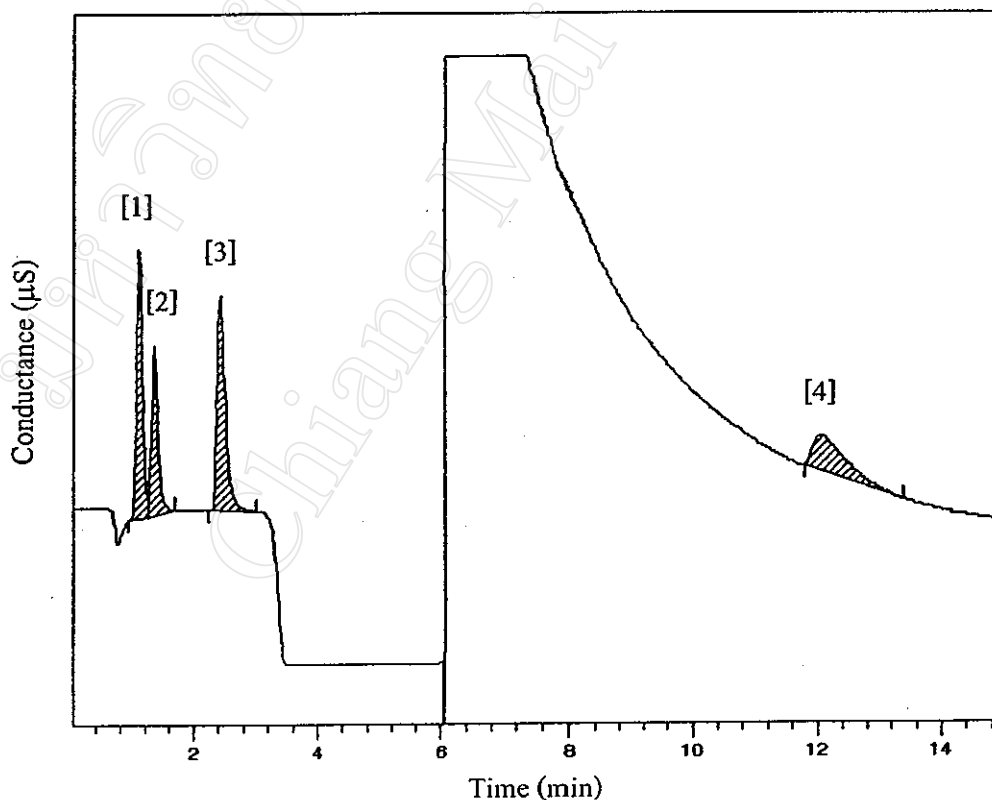
**Figure 3.2** Separation of chlorine-containing anions obtained from the above conditions, [1] chlorite, [2] chloride, [3] chlorate and [4] perchlorate.

Column	IonPac AS4A-SC (4×250) analytical column IonPac AS4A-SC (4×50) guard column
Eluents	3.0 mM sodium hydrogencarbonate [1] and 12.8 mM sodium carbonate[2]
Condition	time 0.0 min %[1] 100 time 2.5 – 2.8 min %[1] 100 to %[2] 100 time 2.8 min %[2] 100
Flow rate	2.00 ml/min
Detector	Conductivity detector, anion self-regenerating suppressor ASRS-1 (4mm) at 100 mA auto-suppression recycle mode



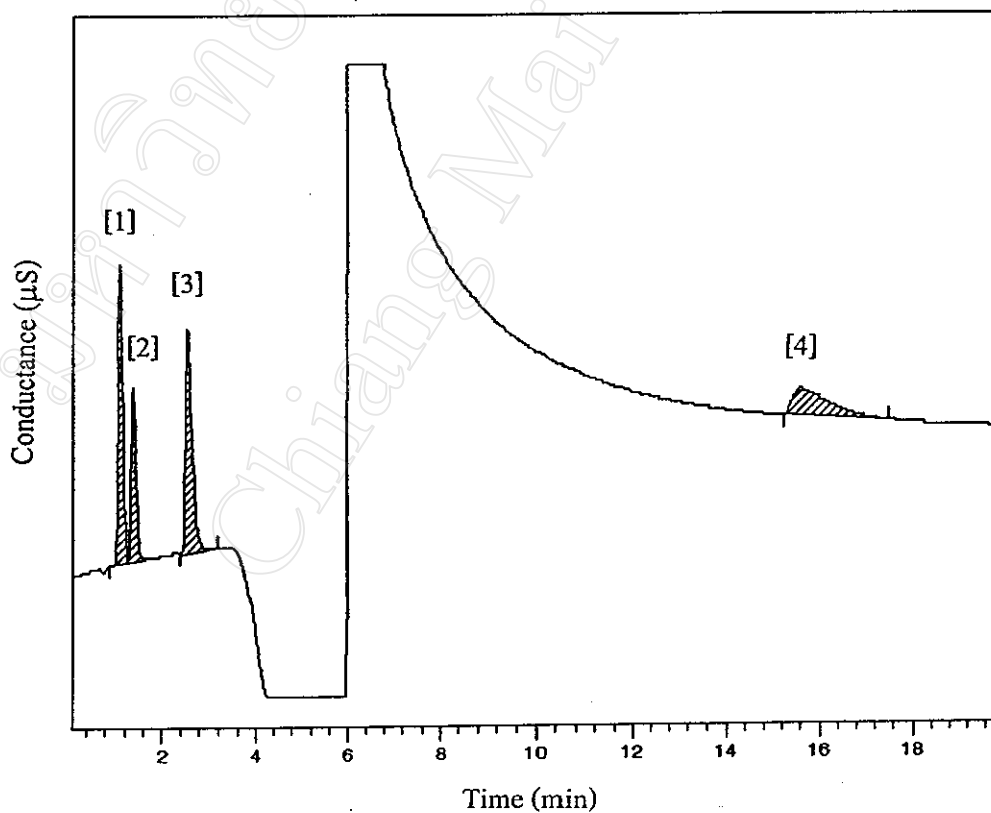
**Figure 3.3** Separation of chlorine-containing anions obtained from the above conditions, [1] chlorite, [2] chloride, [3] chlorate and [4] perchlorate.

Column	IonPac AS4A-SC (4×250) analytical column IonPac AS4A-SC (4×50) guard column
Eluents	3.0 mM sodium benzoate [1] and 0.59 mM sodium citrate[2]
Condition	time 0.0 min %[1] 100 time 1.8 – 2.0 min %[1] 100 to %[2] 100 time 2.0 min %[2] 100
Flow rate	2.00 ml/min
Detector	Conductivity detector, anion self-regenerating suppressor ASRS-1 (4mm) at 100 mA auto-suppression recycle mode



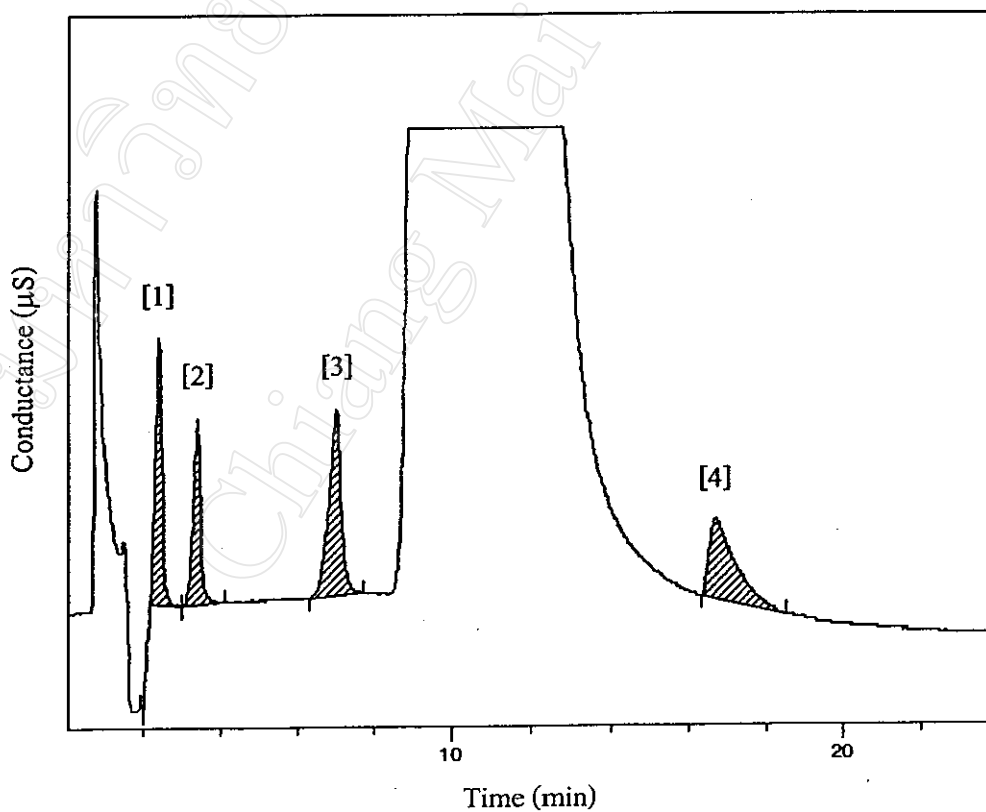
**Figure 3.4** Separation of chlorine-containing anions obtained from the above conditions, [1] chlorite, [2] chloride, [3] chlorate and [4] perchlorate.

Column	IonPac AS4A-SC (4×250) analytical column IonPac AS4A-SC (4×50) guard column
Eluents	1.0 mM sodium benzoate [1] and 0.28 mM sodium citrate[2]
Condition	time 0.0 min %[1] 100 time 2.0 – 2.5 min %[1] 100 to %[2] 100 time 2.5 min %[2] 100
Flow rate	2.00 ml/min
Detector	Conductivity detector, anion self-regenerating suppressor ASRS-1 (4mm) at 100 mA auto-suppression recycle mode



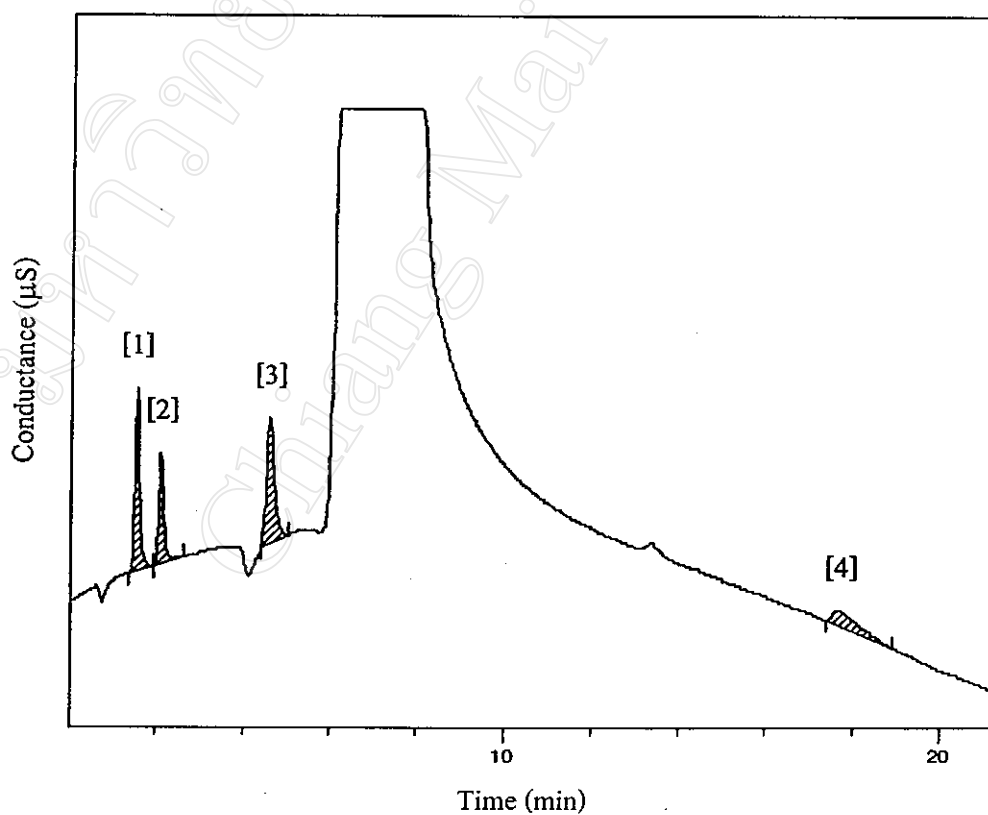
**Figure 3.5** Separation of chlorine-containing anions obtained from the above conditions, [1] chlorite, [2] chloride, [3] chlorate and [4] perchlorate.

Column	IonPac AS4A-SC (4×250) analytical column IonPac AS4A-SC (4×50) guard column
Eluents	1.0 mM citric acid [1] and 1.0 mM sodium citrate[2]
Condition	time 0.0 min %[1] 100 time 7.0 – 8.0 min %[1] 100 to %[2] 100 time 8.0 min %[2] 100
Flow rate	2.00 ml/min
Detector	Conductivity detector, anion self-regenerating suppressor ASRS-1 (4mm) at 100 mA auto-suppression recycle mode



**Figure 3.6** Separation of chlorine-containing anions obtained from the above conditions, [1] chlorite, [2] chloride, [3] chlorate and [4] perchlorate.

Column	IonPac AS4A-SC (4×250) analytical column IonPac AS4A-SC (4×50) guard column
Eluents	0.2 mM sodium benzoate [1] and 12.8 mM sodium carbonate[2]
Condition	time 0.0 min %[1] 100 time 4.5 – 5.0 min %[1] 100 to %[2] 100 time 5.0 min %[2] 100
Flow rate	2.00 ml/min
Detector	Conductivity detector, anion self-regenerating suppressor ASRS-1 (4mm) at 100 mA auto-suppression recycle mode



**Figure 3.7** Separation of chlorine-containing anions obtained from the above conditions, [1] chlorite, [2] chloride, [3] chlorate and [4] perchlorate.

### 3.1.3 Optimization of conditions for a Metrosep A Supp 4 column

#### 3.1.3.1 The effects of the eluent type

The effects of the eluent type were investigated at a flow rate of 1.80 ml/min. The results of retention time, resolution and peak area are shown in Tables 3.8-3.9 and Figure 3.8, respectively.

#### 3.1.3.2 The effects of the concentration of sodium carbonate

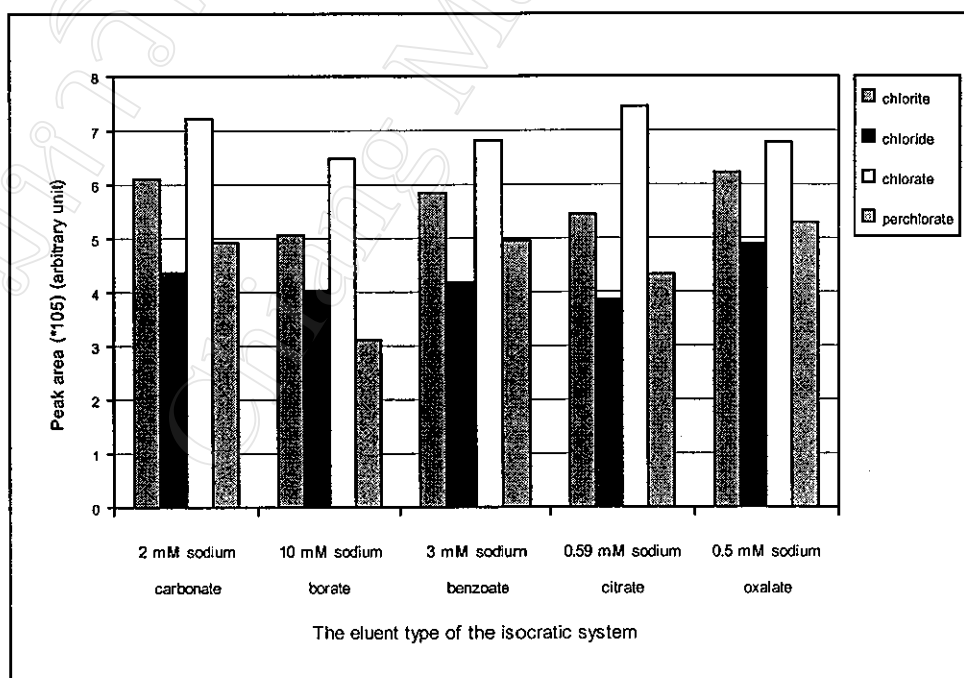
The effects of the concentration of sodium carbonate were investigated at a flow rate of 1.80 ml/min. The results of retention time, resolution and peak area are shown in Tables 3.10-3.11 and Figure 3.9, respectively.

**Table 3.8** Effects of eluent type on retention time of chlorine-containing anions with a Metrosep A Supp 4 column at flow rate of 1.80 ml/min

Eluent type	Average retention time (min)			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
2 mM sodium carbonate	2.60	2.93	4.00	20.26
10 mM sodium borate	1.97	2.22	3.23	21.67
3 mM sodium benzoate	5.40	6.30	9.53	67.50
0.59 mM sodium citrate	2.00	2.22	2.93	14.25
0.5 mM sodium oxalate	4.78	5.70	9.67	78.40
acetonitrile	NF	NF	NF	NF

**Table 3.9** Effects of eluent type on resolution of chlorine-containing anions with a Metrosep A Supp 4 column at flow rate of 1.80 ml/min

Eluent type	Average resolution		
	$\text{ClO}_2^- - \text{Cl}^-$	$\text{Cl}^- - \text{ClO}_3^-$	$\text{ClO}_3^- - \text{ClO}_4^-$
2 mM sodium carbonate	1.74	4.80	16.07
10 mM sodium borate	1.41	4.52	9.83
3 mM sodium benzoate	1.09	7.80	23.52
0.59 mM sodium citrate	1.27	3.62	16.62
0.5 mM sodium oxalate	3.12	9.53	19.01
acetonitrile	-	-	-



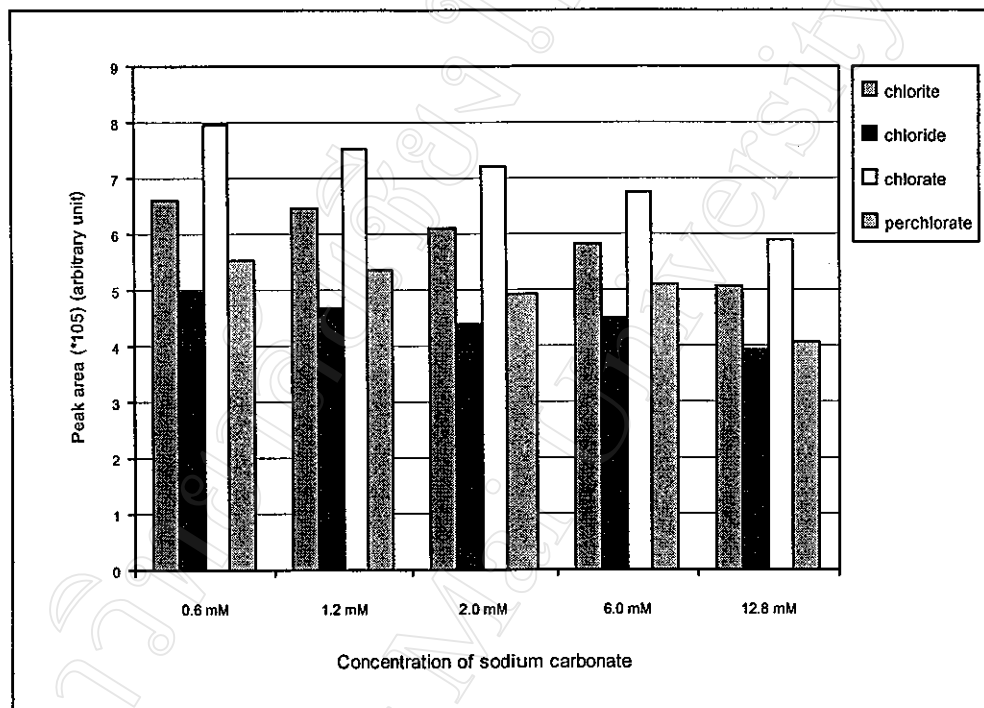
**Figure 3.8** Effects of eluent type on peak area of chlorine-containing anions with a Metrosep A Supp 4 column at flow rate of 1.80 ml/min.

**Table 3.10** Effects of concentration of sodium carbonate on retention time of chlorine-containing anions with a Metrosep A Supp 4 column at flow rate of 1.80 ml/min

Eluent type	Average retention time (min)			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
0.6 mM sodium carbonate	4.39	5.07	7.21	39.71
1.2 mM sodium carbonate	3.18	3.62	5.00	25.38
2.0 mM sodium carbonate	2.60	2.93	4.00	20.20
6.0 mM sodium carbonate	1.88	2.08	2.73	12.85
12.8 mM sodium carbonate	1.72	1.86	2.39	11.06

**Table 3.11** Effects of concentration of sodium carbonate on resolution of chlorine-containing anions with a Metrosep A Supp 4 column at flow rate of 1.80 ml/min

Eluent type	Average resolution		
	$\text{ClO}_2^- - \text{Cl}^-$	$\text{Cl}^- - \text{ClO}_3^-$	$\text{ClO}_3^- - \text{ClO}_4^-$
0.6 mM sodium carbonate	2.62	6.72	18.23
1.2 mM sodium carbonate	2.13	5.54	17.24
2.0 mM sodium carbonate	1.74	4.80	16.07
6.0 mM sodium carbonate	1.15	3.41	14.63
12.8 mM sodium carbonate	0.64	2.68	13.48



**Figure 3.9** Effects of concentration of sodium carbonate on peak area of chlorine-containing anions with a Metrosep A Supp 4 column at flow rate of 1.80 ml/min.

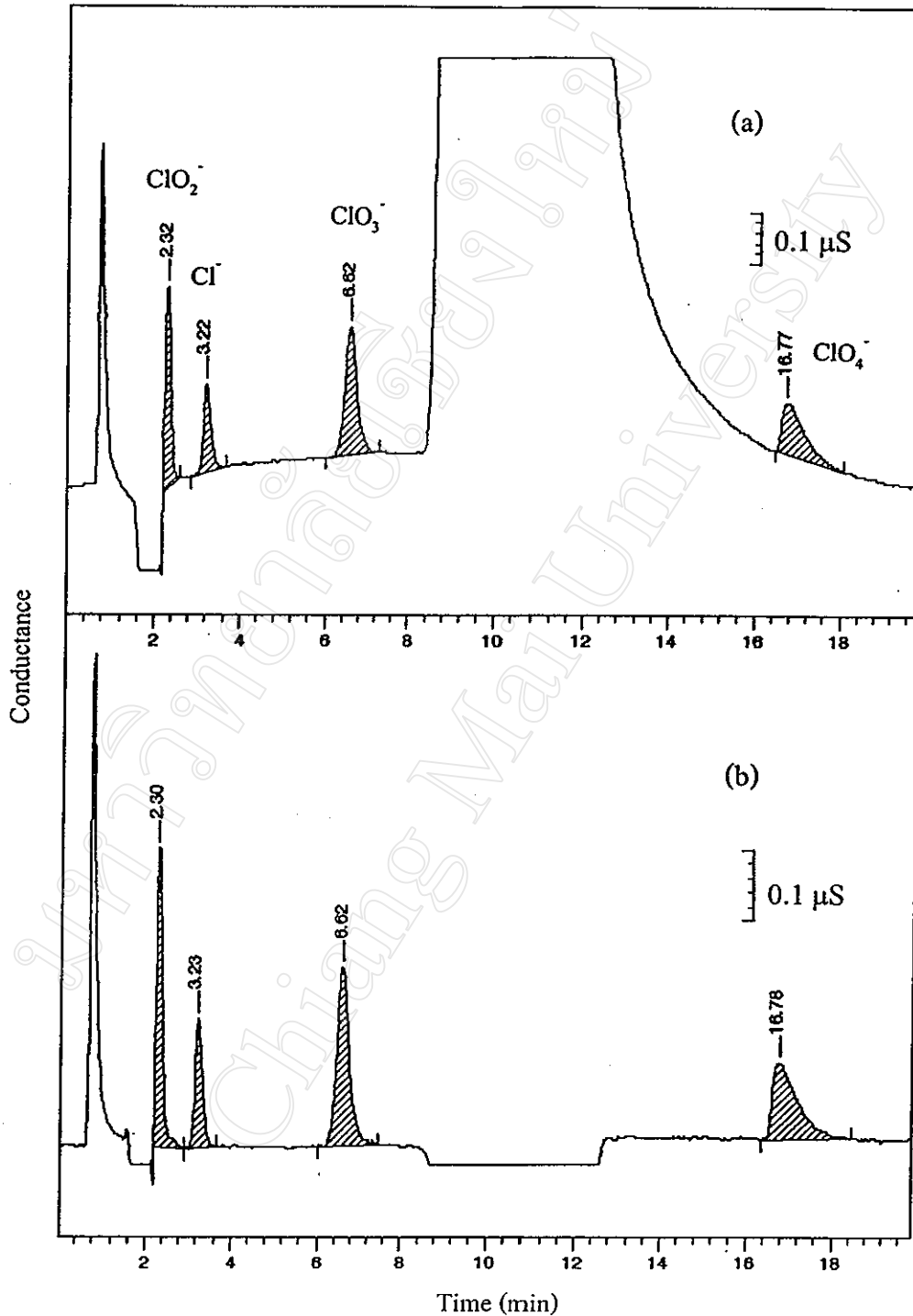
### 3.2 Optimal Conditions for Chlorine-containing Anions

#### 3.2.1 Gradient conditions obtained with an IonPac AS4A-SC column

The optimal conditions for analysis of chlorine-containing anions, obtained with an IonPac AS4A-SC anion-exchange column can be summarized in Table 3.12. The chromatograms of chlorine-containing anions under the gradient conditions are presented in Figure 3.10.

**Table 3.12** The gradient conditions obtained with an IonPac AS4A-SC column for the separation of chlorine-containing anions

Operation	conditions
Eluent	1.0 mM citric acid [1] and 1.0 mM sodium citrate[2]
Condition	time 0.0 min %[1] 100 time 7.0 – 8.0 min %[1] 100 to %[2] 100 time 8.0 min %[2] 100
Eluent flow rate	2.00 ml/min
Injection volume	100 $\mu$ l
Detector	conductivity
Suppression	anion self-regenerating suppressor (auto-suppression recycle mode)
Background conductivity	223 $\mu$ S
Detector temperature compensation	1.7 %/ $^{\circ}$ C



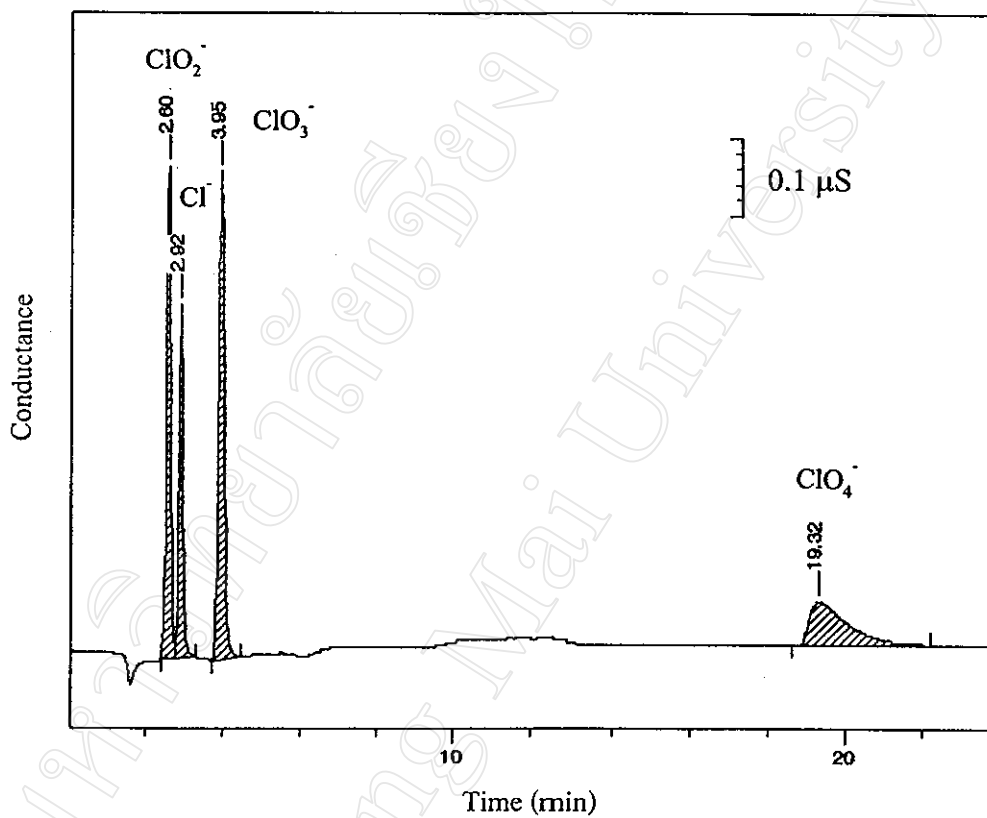
**Figure 3.10** Chromatograms of chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector using the gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min: (a) normal integration (b) subtract integration.

### 3.2.2 Isocratic conditions obtained with a Metrosep A Supp 4 column

The optimal conditions for analysis of chlorine-containing anions, obtained with a Metrosep A Supp 4 anion-exchange column can be summarized in Table 3.13. The chromatogram of the isocratic conditions is presented in Figure 3.11.

**Table 3.13** The isocratic conditions obtained with a Metrosep A Supp 4 column for the separation of chlorine-containing anions

Operation	conditions
Eluent	2.0 mM sodium carbonate
Eluent flow rate	1.80 ml/min
Injection volume	100 $\mu$ l
Detector	conductivity
Suppression	anion self-regenerating suppressor (auto-suppression recycle mode)
Background conductivity	20 $\mu$ S
Detector temperature compensation	1.7 %/ $^{\circ}$ C



**Figure 3.11** Chromatogram of chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using the isocratic eluent, 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.

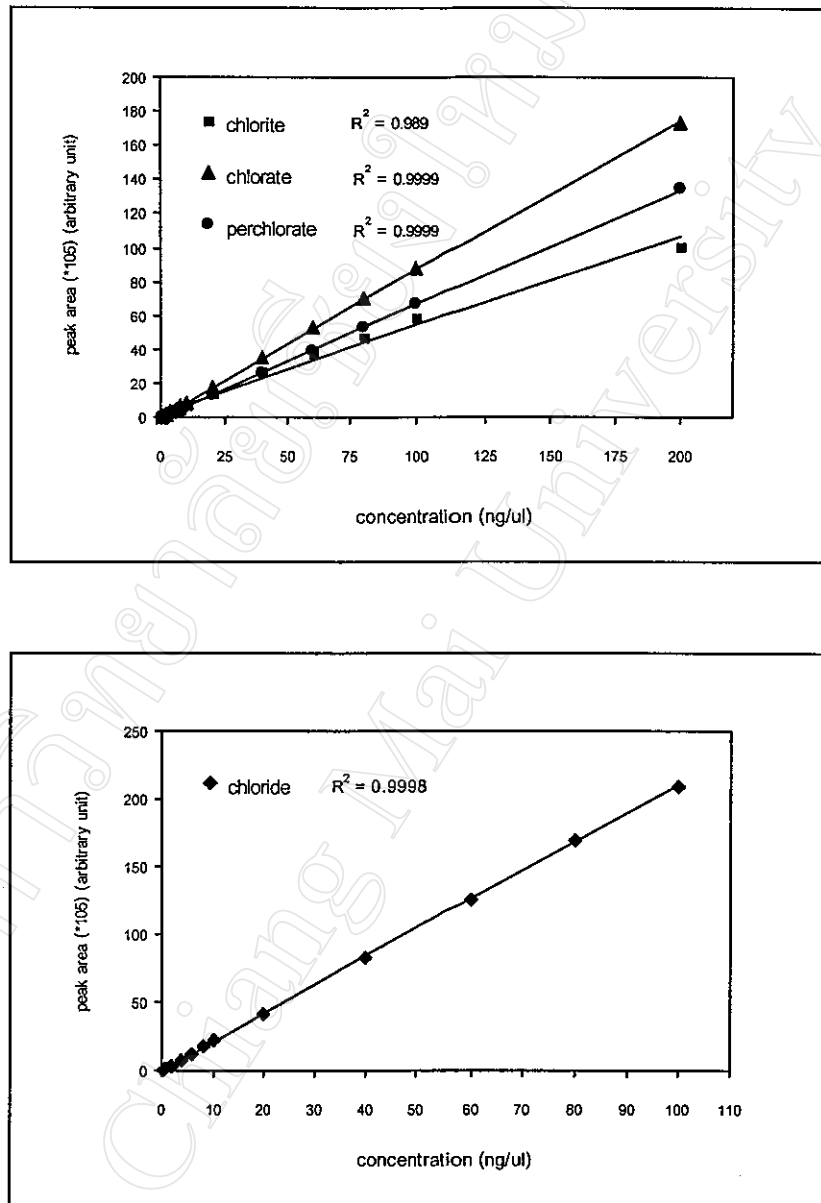
### 3.3 Gradient System Using an IonPac AS4A-SC Anion-exchange Column

#### 3.3.1 Linearity of gradient system

The concentrations of chlorite, chloride, chlorate and perchlorate were investigated in the range of 0.2-200.0 ng/ $\mu$ l. The obtained results of average peak area and external calibration curves are shown in Table 3.14 and Figure 3.12, respectively.

**Table 3.14** Average peak areas (n=3) corresponding to the concentration of chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector using the gradient eluents at flow rate of 2.00 ml/min

Concentration (ng/ $\mu$ l)	Average peak area ( $\times 10^5$ ) (arbitrary unit)			
	ClO <sub>2</sub> <sup>-</sup>	Cl <sup>-</sup>	ClO <sub>3</sub> <sup>-</sup>	ClO <sub>4</sub> <sup>-</sup>
0.2	-	0.22	0.22	-
0.4	0.22	0.69	0.32	0.11
0.8	0.40	1.74	0.64	0.32
2.0	1.05	3.66	1.62	1.10
4.0	2.71	7.67	3.32	2.38
6.0	3.95	12.38	5.10	3.56
8.0	5.22	17.82	6.78	4.91
10.0	6.85	21.89	8.47	6.18
20.0	13.75	40.79	17.30	12.68
40.0	27.11	82.80	35.60	25.92
60.0	37.99	124.67	52.54	39.11
80.0	46.77	169.53	70.57	52.59
100.0	58.68	209.52	88.21	66.15
200.0	100.47	-	178.09	134.18



**Figure 3.12** The calibration curves for chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.

### 3.3.2 Reproducibility of gradient system

The seven replicates of chlorine-containing anions solution were introduced onto the IonPac AS4A-SC column under the established condition. The relative standard deviation values of retention time and peak area of each chlorine-containing anion are shown in Tables 3.15 and 3.16, respectively.

**Table 3.15** Reproducibility of retention time (n=3) of chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector with gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min

Run	Average retention time (min)			
	ClO <sub>2</sub> <sup>-</sup> (8.0 ng/μl)	Cl <sup>-</sup> (0.8 ng/μl)	ClO <sub>3</sub> <sup>-</sup> (8.0 ng/μl)	ClO <sub>4</sub> <sup>-</sup> (8.0 ng/μl)
1	2.35	3.27	6.68	16.63
2	2.32	3.25	6.65	16.62
3	2.35	3.27	6.68	16.62
4	2.35	3.27	6.68	16.58
5	2.32	3.23	6.62	16.52
6	2.33	3.25	6.65	16.53
7	2.33	3.25	6.65	16.53
mean	2.34	3.26	6.66	16.58
SD	0.01	0.02	0.02	0.05
%RSD	0.43	0.61	0.30	0.30

**Table 3.16** Reproducibility of peak area (n=3) of chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector with gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min

Run	Average retention time (min)			
	ClO <sub>2</sub> <sup>-</sup> (8.0 ng/μl)	Cl <sup>-</sup> (0.8 ng/μl)	ClO <sub>3</sub> <sup>-</sup> (8.0 ng/μl)	ClO <sub>4</sub> <sup>-</sup> (8.0 ng/μl)
1	4.98	2.71	6.82	4.92
2	5.14	2.62	6.74	4.81
3	5.02	2.53	6.70	4.93
4	5.48	2.77	6.90	4.99
5	5.06	2.63	6.92	4.93
6	5.12	2.55	6.73	5.00
7	5.41	2.56	6.69	4.97
mean	5.17	2.62	6.79	4.94
SD	0.19	0.09	0.09	0.06
%RSD	3.68	3.44	1.33	1.21

### 3.3.3 Detection limit of gradient system

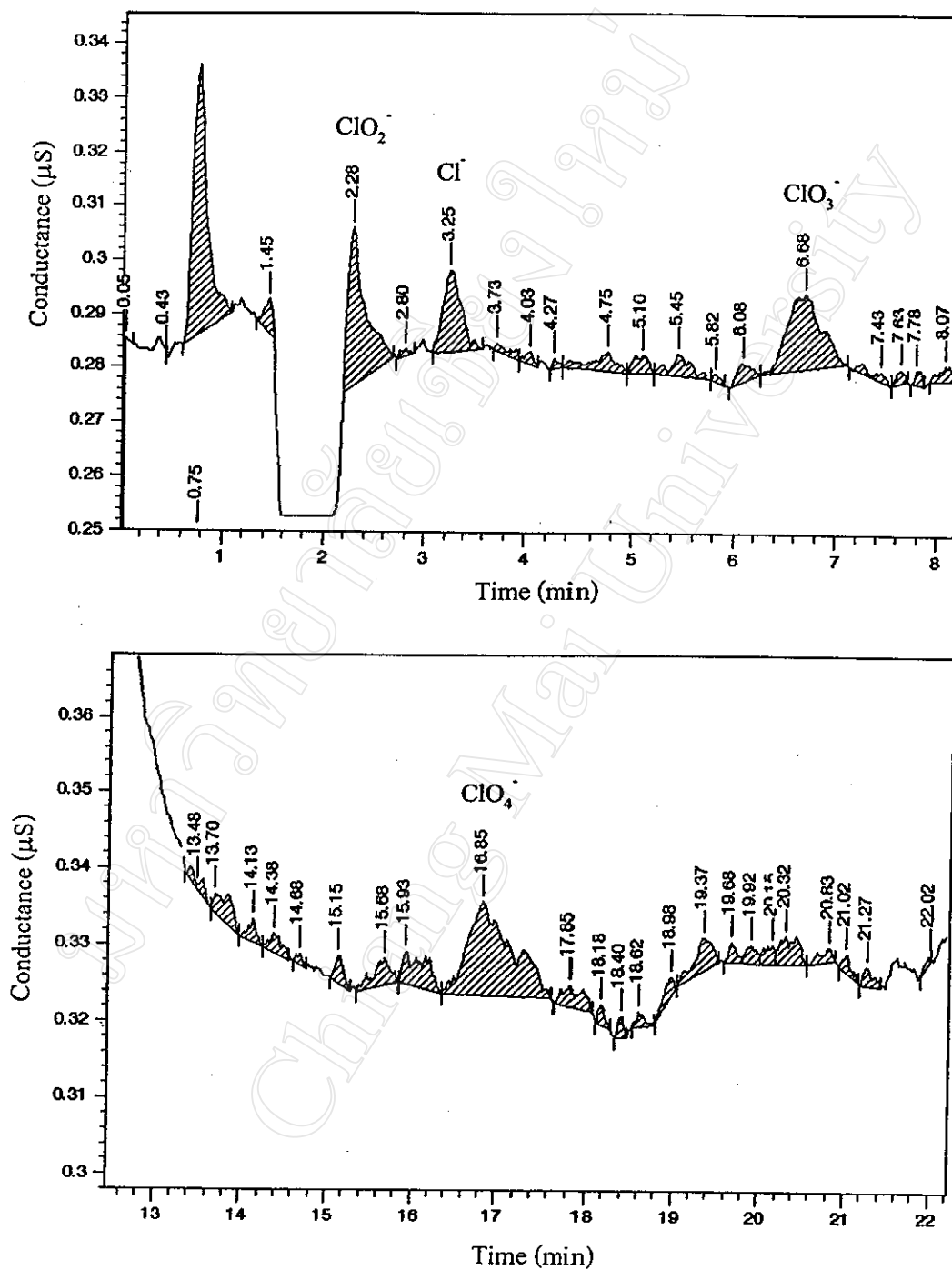
The lowest concentration of chlorite, chloride, chlorate and perchlorate was used for determination of detection limit. The obtained results and the chromatograms of chlorine-containing anions are shown in Tables 3.17-3.18 and Figure 3.13, respectively.

**Table 3.17** The data of noise level (n), peak signal response (R) and width at half height of peak ( $w_{0.5}$ ) of chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min

Data	Response of chlorine-containing anions			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_4^-$	$\text{ClO}_4^-$
Height of noise level ( $\mu\text{S}$ )	0.0009	0.0104	0.0006	0.0016
	0.0007	0.0116	0.0013	0.0015
	0.0006	0.0129	0.0015	0.0020
mean	0.0007	0.0116	0.0011	0.0017
Height of peak signal response ( $\mu\text{S}$ )	0.0028	0.0203	0.0037	0.0018
	0.0027	0.0213	0.0032	0.0023
	0.0024	0.0191	0.0033	0.0027
	0.0022	0.1800	0.0055	0.0036
	0.0023	0.0227	0.0053	0.0022
	0.0035	0.0231	0.0061	0.0031
	0.0023	0.0234	0.0054	0.0033
Mean	0.0026	0.0211	0.0046	0.0027
Width at half height of peak (sec)	0.135	0.110	0.244	0.263
	0.179	0.128	0.246	0.106
	0.078	0.105	0.275	0.248
	0.151	0.113	0.402	0.222
	0.196	0.116	0.482	0.300
	0.143	0.127	0.247	0.177
	0.123	0.126	0.276	0.108
Mean	0.144	0.118	0.310	0.203

**Table 3.18** The detection limit (L) and minimum detectable quantity (MDQ) of chlorine-containing anions obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min

Chlorine-containing anions	Concentration (ng/ $\mu$ l)	Detection limit (L) (ng)	Minimum detectable quantity (MDQ) ( $\times 10^{-2}$ )(ng.sec)
$\text{ClO}_2^-$	0.05	0.03	0.43
$\text{Cl}^-$	0.01	0.01	0.12
$\text{ClO}_3^-$	0.05	0.02	0.62
$\text{ClO}_4^-$	0.05	0.06	1.22



**Figure 3.13** Chromatograms of chlorine-containing anions at lowest concentration, showing the peak signal response ( $R$ ) and width at half height of peak ( $w_{0.5}$ ) using an IonPac AS4A-SC column and a conductivity detector with gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.

### 3.3.4 Recovery of chlorine-containing anions in the water samples using gradient system

The accuracy of quantification of chlorine-containing anions with the IonPac AS4A-SC column was improved by the spiked recovery method. The water samples were selected for this investigation consisting of (1) Maharaj Nakorn Chiang Mai Hospital, (2) Rujirawong swimming pool in Chiang Mai University and (3) Mae-Ping river. The obtained results of %recoveries of each chlorine-containing anion are shown in Tables 3.19-3.22 and the chromatograms of the spiked water samples and non-spiked water samples are presented in Figures 3.14 and 3.15.

**Table 3.19** The percentage recovery of chlorite in the water samples with the spiked recovery method at various concentrations using gradient system

Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	4.0	195909	3.44	86.00
	8.0	419256	6.76	84.50
	16.0	835818	13.97	81.06
Rujirawong swimming pool water (Sample No.11)	4.0	215222	3.72	93.00
	8.0	412478	6.66	83.25
	16.0	881096	13.64	85.25
Natural water in Mae-Ping river (Sample No.15)	4.0	204566	3.57	89.25
	8.0	404557	6.55	81.88
	16.0	871945	13.50	84.38
Recovery range		81.06-93.00% (mean 85.40%)		
SD		3.77		
%RSD		4.41%		

**Table 3.20** The percentage recovery of chloride in the water samples with the spiked recovery method at various concentrations using gradient system

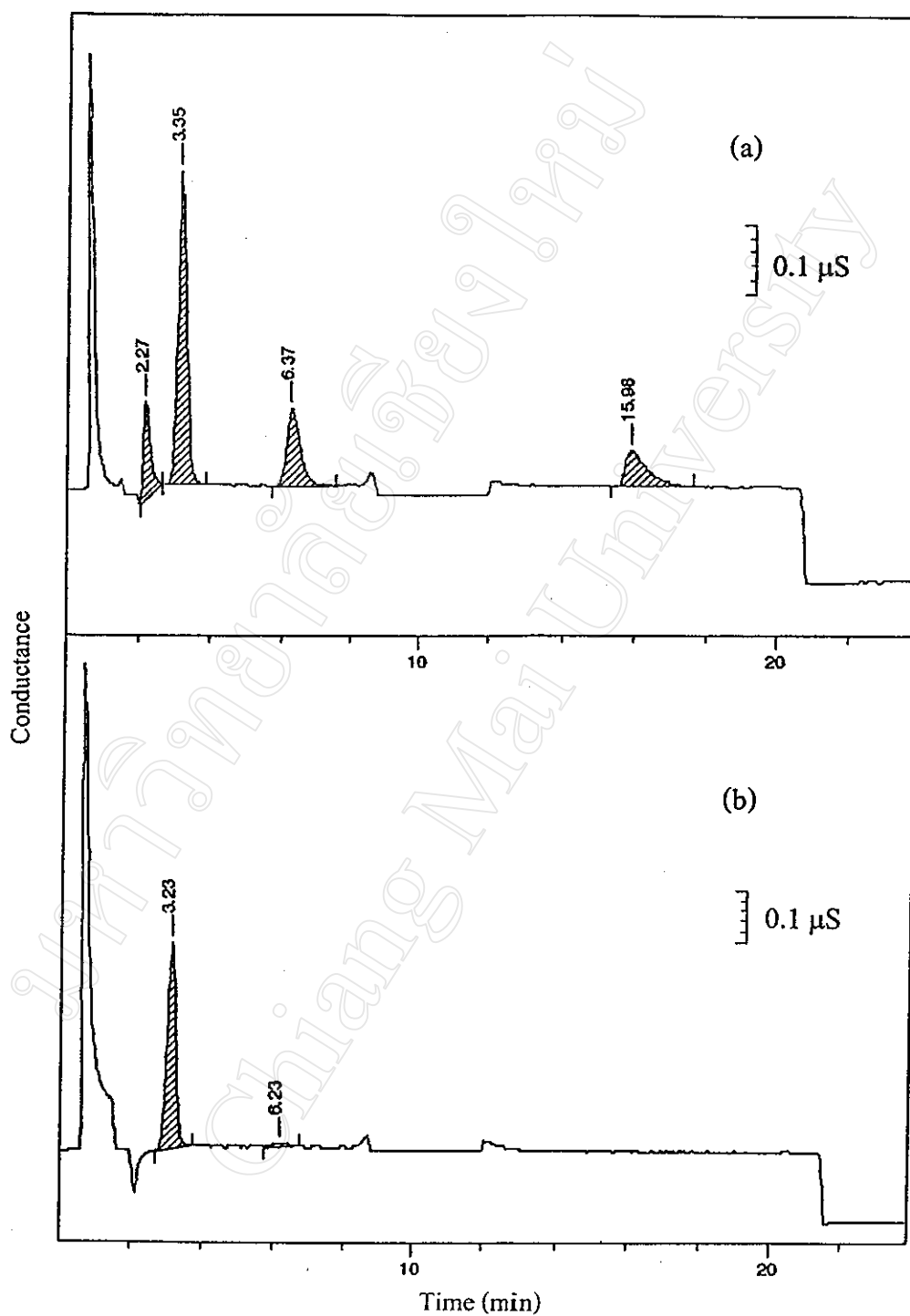
Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	4.0	911779	4.02	100.50
	8.0	2166427	9.56	119.50
	16.0	4121747	18.19	113.69
Rujirawong swimming pool water (Sample No.11)	4.0	1005876	4.43	110.75
	8.0	2091006	9.22	115.25
	16.0	4092752	18.05	112.81
Natural water in Mae-Ping river (Sample No.15)	4.0	1092453	4.83	120.75
	8.0	2098099	9.26	115.75
	16.0	3904276	17.23	107.69
Recovery range		100.50-120.75% (mean 112.97%)		
SD		6.17		
%RSD		5.46%		

**Table 3.21** The percentage recovery of chlorate in the water samples with the spiked recovery method at various concentrations using gradient system

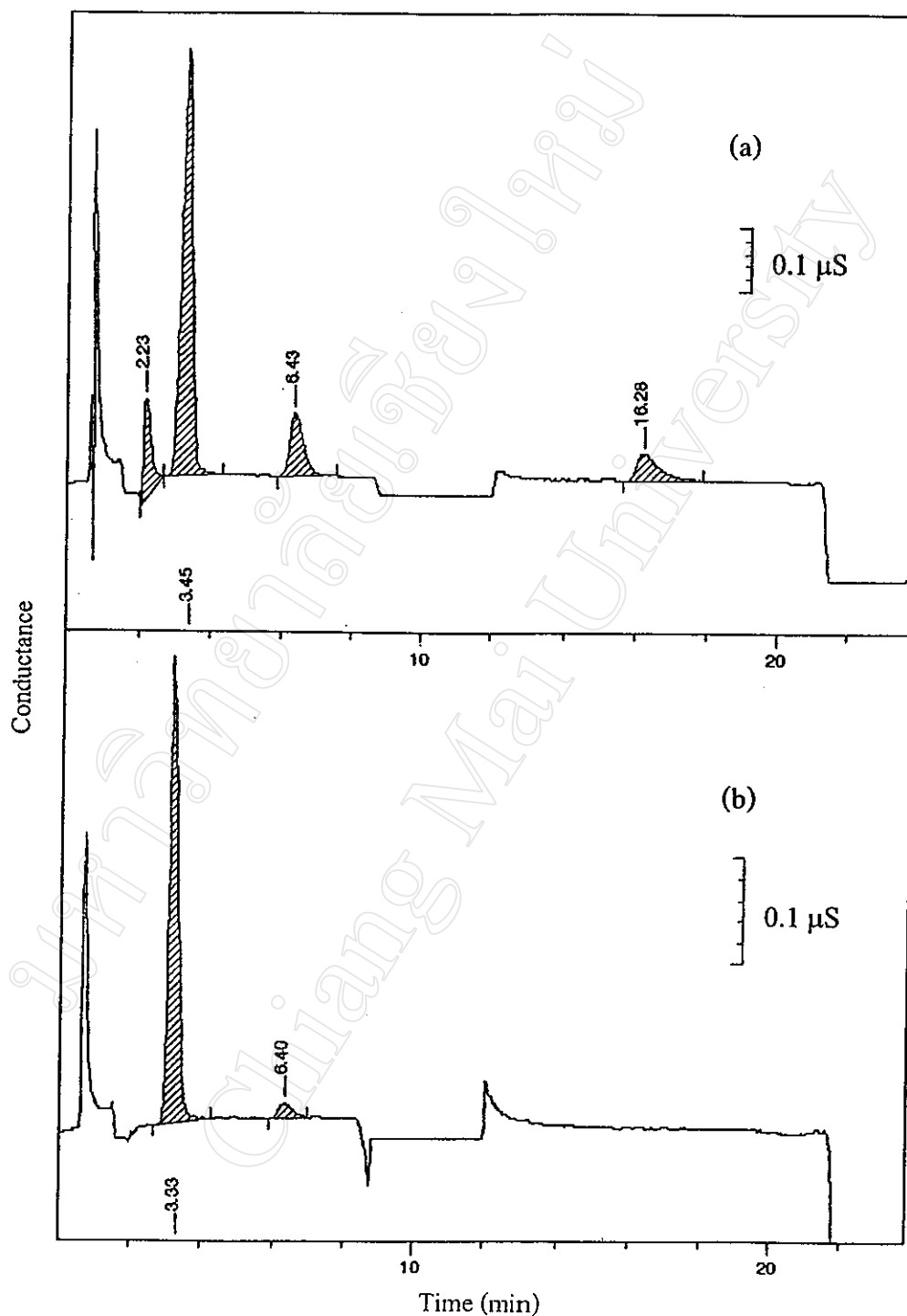
Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	4.0	369431	4.29	85.00
	8.0	698691	8.02	89.13
	16.0	1346235	15.33	90.25
Rujirawong swimming pool water (Sample No.11)	4.0	446797	5.17	87.00
	8.0	739911	8.48	84.88
	16.0	1306519	14.88	82.44
Natural water in Mae-Ping river (Sample No.15)	4.0	292286	3.42	85.50
	8.0	573848	6.61	82.63
	16.0	1212123	13.82	86.38
Recovery range		82.44-90.25% (mean 85.91%)		
SD		2.63		
%RSD		3.06%		

**Table 3.22** The percentage recovery of perchlorate in the water samples with the spiked recovery method at various concentrations using gradient system

Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	4.0	237496	3.89	97.25
	8.0	497486	7.93	99.13
	16.0	1003299	15.80	98.78
Rujirawong swimming pool water (Sample No.11)	4.0	246425	4.03	100.75
	8.0	502029	8.01	100.13
	16.0	999584	15.76	98.50
Natural water in Mae-Ping river (Sample No.15)	4.0	242602	3.98	99.50
	8.0	501563	8.01	100.13
	16.0	1007697	15.88	99.25
Recovery range		97.25-100.75% (mean 99.27%)		
SD		1.04		
%RSD		1.05%		



**Figure 3.14** The chromatograms of (a) the spiked and (b) non-spiked samples of public water supply in Maharaj Nakorn Chiang Mai Hospital obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.

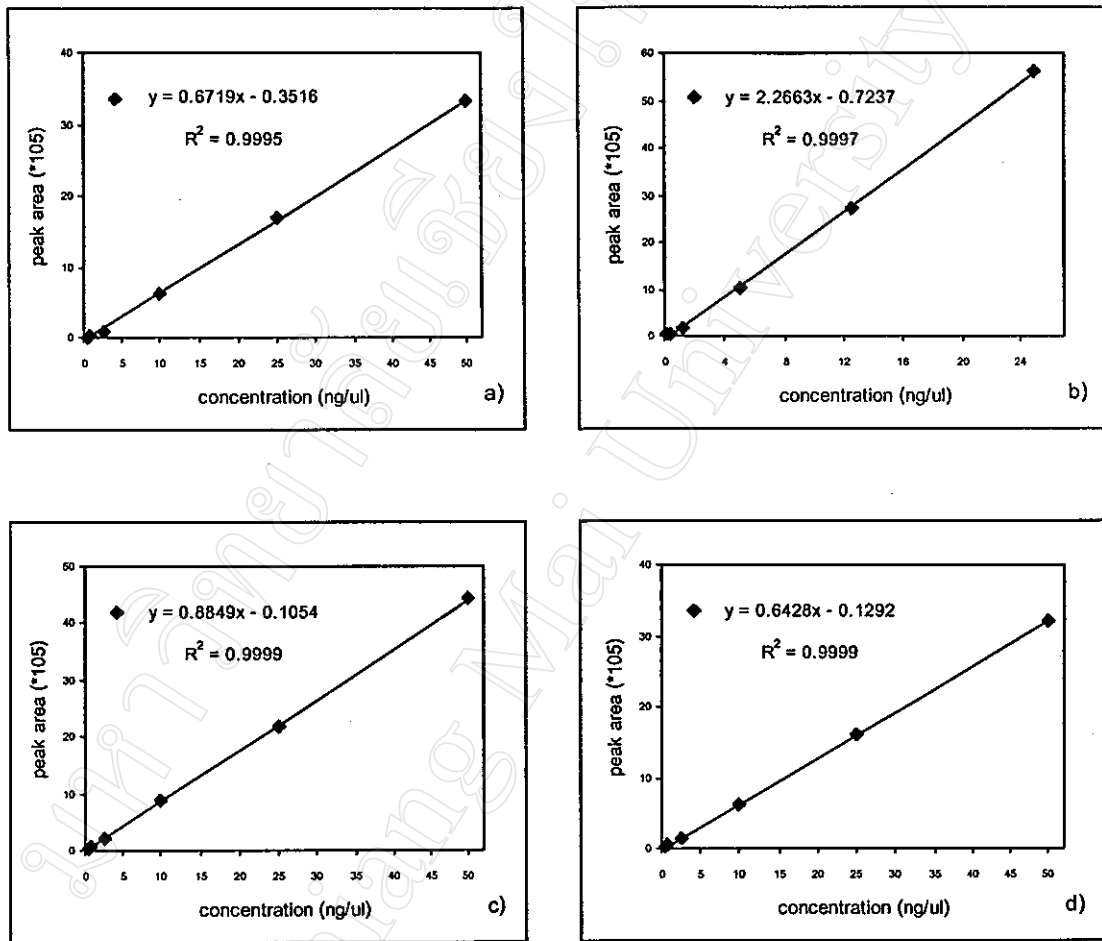


**Figure 3.15** The chromatograms of (a) the spiked and (b) non-spiked samples of Rujirawong swimming pool water (25 fold dilution) in Chiang Mai University obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, at flow rate of 2.00 ml/min.

### 3.3.5 Determination of chlorine-containing anions in the water samples using gradient system

The external standard method was used in the investigation by the calibration curves of chlorine-containing anions, constructed from average peak area and concentration of chlorite, chlorate and perchlorate in the range of 0.4-50.0 ng/ $\mu$ l and chloride in the range of 0.2-25.0 ng/ $\mu$ l, as shown in Figure 3.16.

The determination of chlorine-containing anions in the water samples was carried out using the gradient system obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, 1.0 mM citric acid and 1.0 mM sodium citrate at flow rate of 2.00 ml/min. From the analysis, the chlorine-containing anions, which were found in the water samples, were chloride and chlorate. The results are shown in Table 3.23 and the chromatograms of some water samples are presented in Figures 3.17-3.20.



**Figure 3.16** The calibration curves of chlorine-containing anions

a) chlorite, b) chloride, c) chlorate and d) perchlorate.

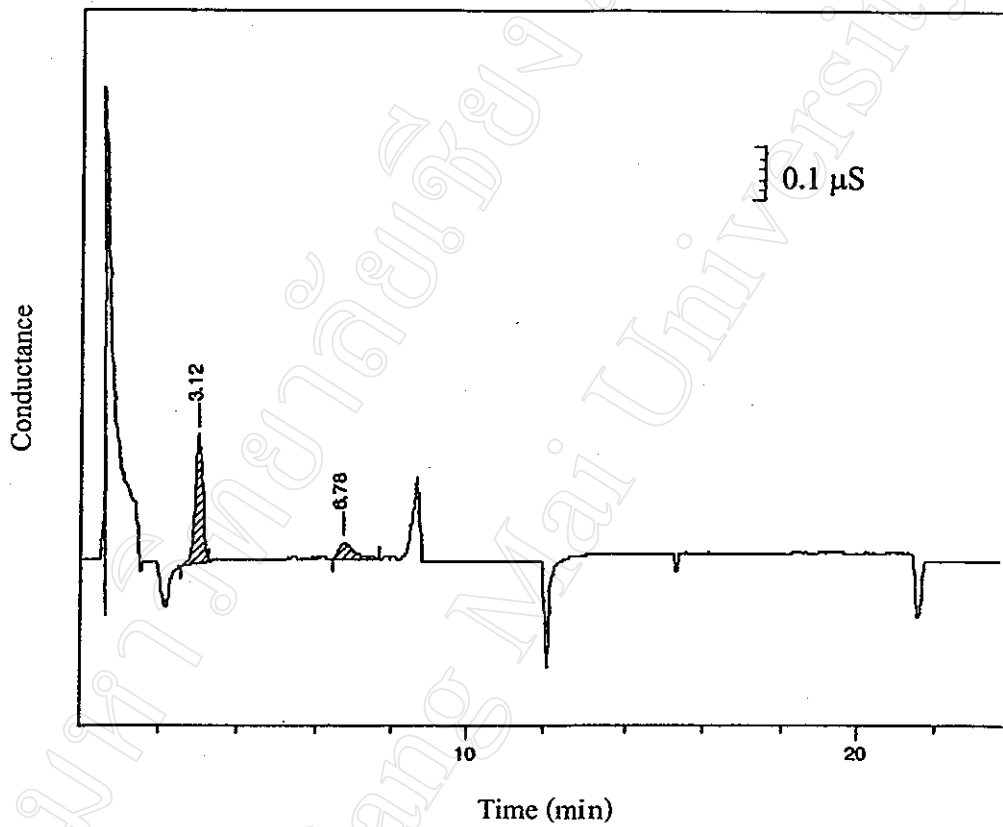
**Table 3.23** The results of determination of chlorine-containing anions in the water samples obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min

Sample No.	Chloride ion		Chlorate ion	
	Average peak area	Concentration (ng/ $\mu$ l)	Average peak area	Concentration (ng/ $\mu$ l)
Sample No.1	1022608	4.83	-	-
Sample No.2	1375770	6.39	-	-
Sample No.3	1171011	5.49	-	-
Sample No.4	953594	4.53	-	-
Sample No.5	1076047	5.07	-	-
Sample No.6	782911	3.77	-	-
Sample No.7	1093299	5.14	-	-
Sample No.8	2568950	11.65	-	-
Sample No.9	4195930	18.83	-	-
Sample No.10	2001158	9.15	67810	0.89
Sample No.11 <sup>a</sup>	2278683	10.38 (259.50 <sup>c</sup> )	138814	1.69 (42.25 <sup>c</sup> )
Sample No.12 <sup>a</sup>	2707969	12.27 (306.75 <sup>c</sup> )	71877	0.93 (23.25 <sup>c</sup> )
Sample No.13 <sup>b</sup>	4653334	20.85 (417.00 <sup>c</sup> )	69735	0.91 (18.20 <sup>c</sup> )
Sample No.14	535868	2.68	-	-
Sample No.15	767324	3.70	-	-

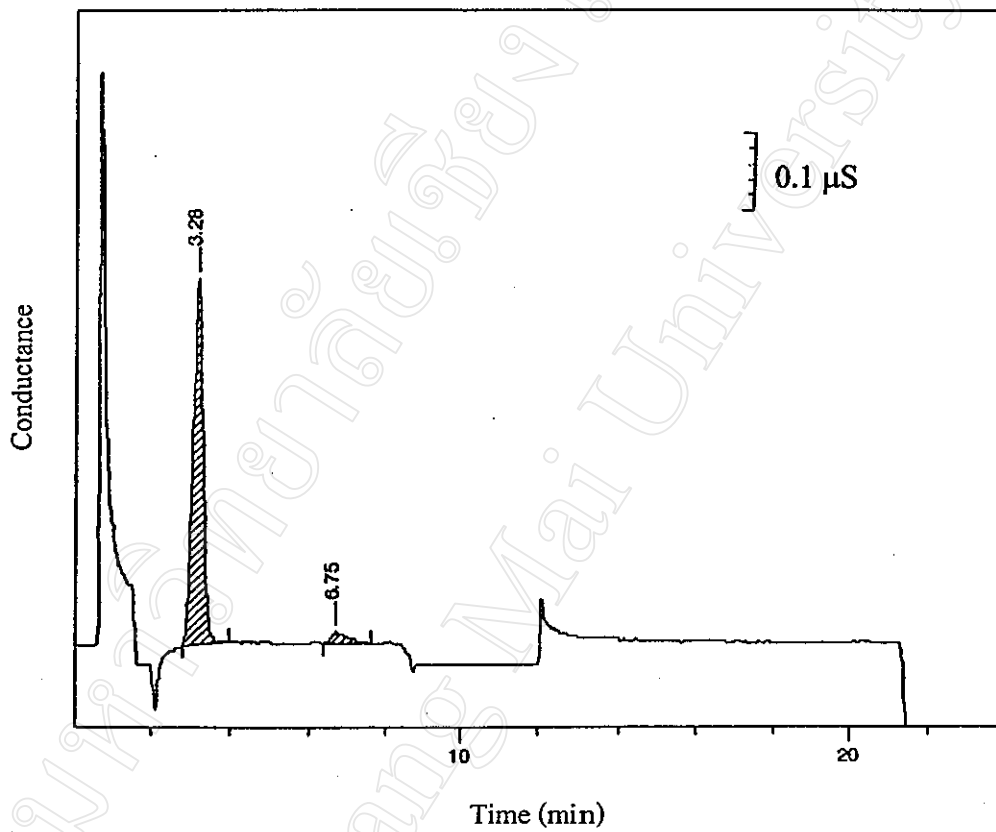
<sup>a</sup> 25 fold dilution

<sup>b</sup> 20 fold dilution

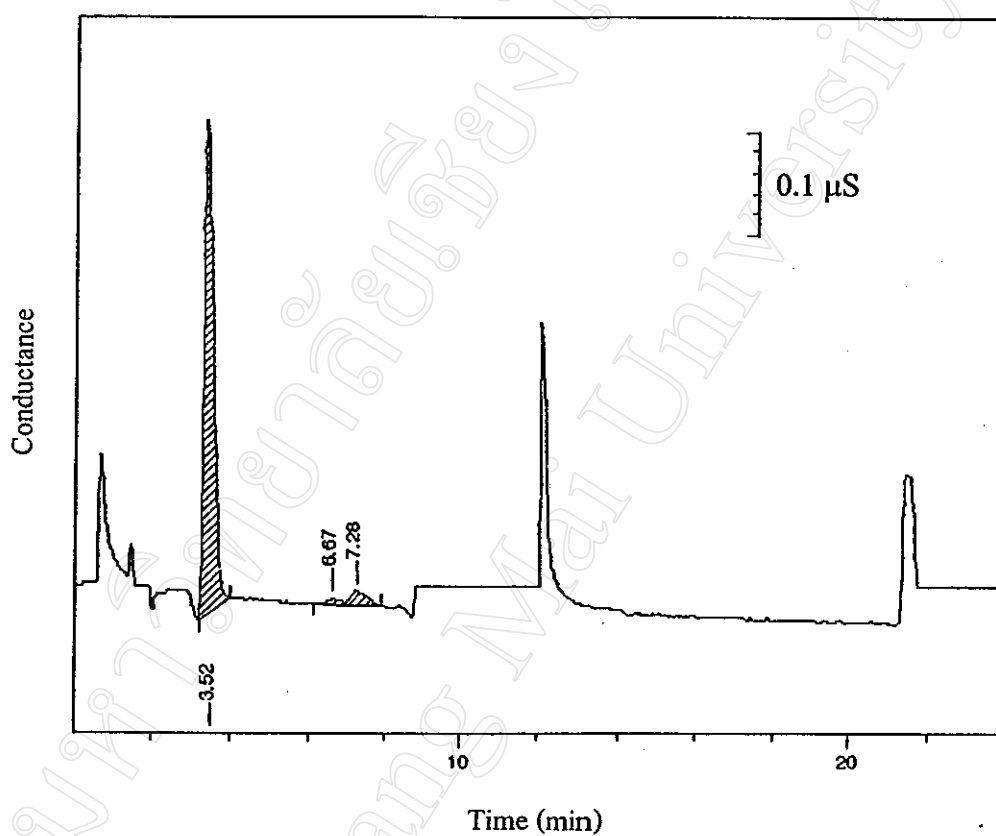
<sup>c</sup> original concentration



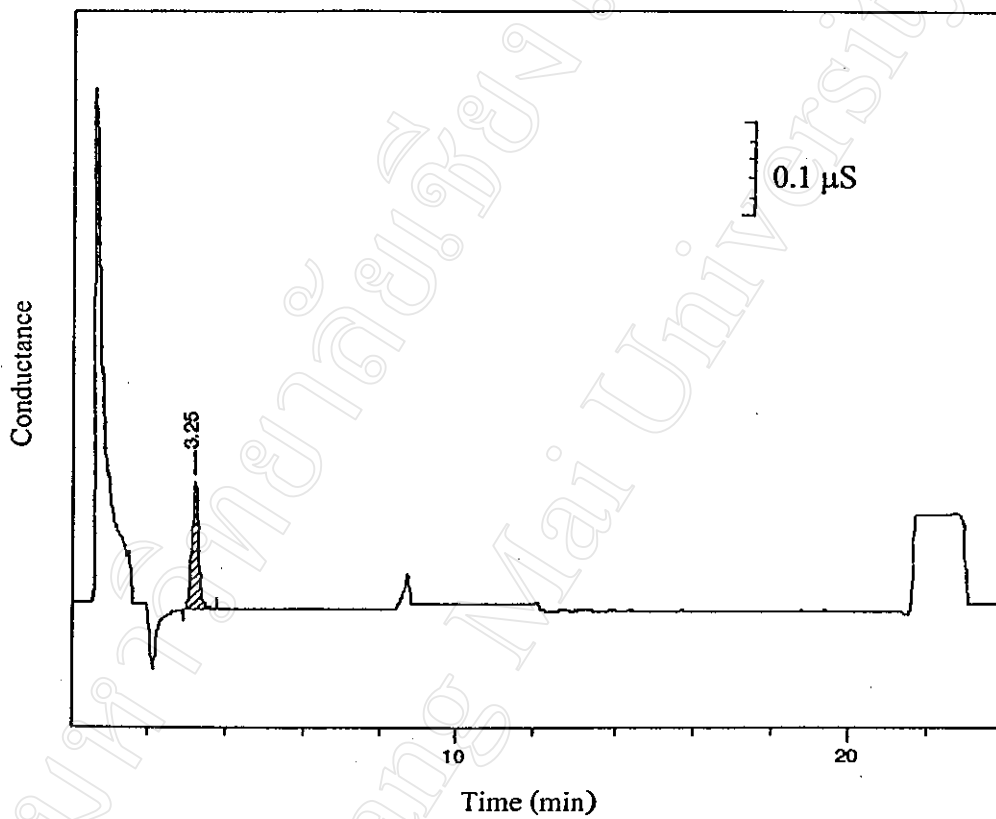
**Figure 3.17** The chromatogram of the natural water in longan orchard (sample No.4) obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.



**Figure 3.18** The chromatogram of the public water supply in Chiang Mai University (sample No.8) obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.



**Figure 3.19** The chromatogram of the swimming pool water (sample No.13, 20 fold dilution) in Amari Rincome Hotel obtained with an IonPac AS4A- SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.



**Figure 3.20** The chromatogram of the natural water of Ang-Kaew reservoir, in Chiang Mai University (sample No.14) obtained with an IonPac AS4A-SC column and a conductivity detector using gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.

### 3.4 Isocratic System Using a Metrosep A Supp 4 Anion-exchange Column

#### 3.4.1 Linearity of isocratic system

The concentrations of chlorine-containing anions were investigated in the range of 0.1-100.0 ng/ $\mu$ l. The results of average peak area and the external calibration curves are shown in Table 3.24 and Figure 3.21, respectively.

**Table 3.24** Average peak areas (n=3) corresponding to the concentrations of chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min

Concentration (ng/ $\mu$ l)	Average peak area ( $\times 10^5$ ) (arbitrary unit)			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
0.1	0.19	0.26	0.12	-
0.2	0.30	0.49	0.25	0.05
0.4	0.68	1.38	0.53	0.27
0.8	1.23	2.55	1.00	0.62
2.0	2.60	6.46	2.69	1.75
4.0	5.20	13.91	5.36	3.46
6.0	7.37	22.56	8.25	5.32
8.0	11.12	31.35	11.31	7.04
10.0	14.14	40.13	14.38	8.95
20.0	31.52	79.58	31.21	18.19
40.0	64.59	165.36	64.16	35.58
60.0	92.56	254.18	98.47	53.36
80.0	132.11	343.24	136.98	70.04
100.0	164.41	427.84	174.42	88.89

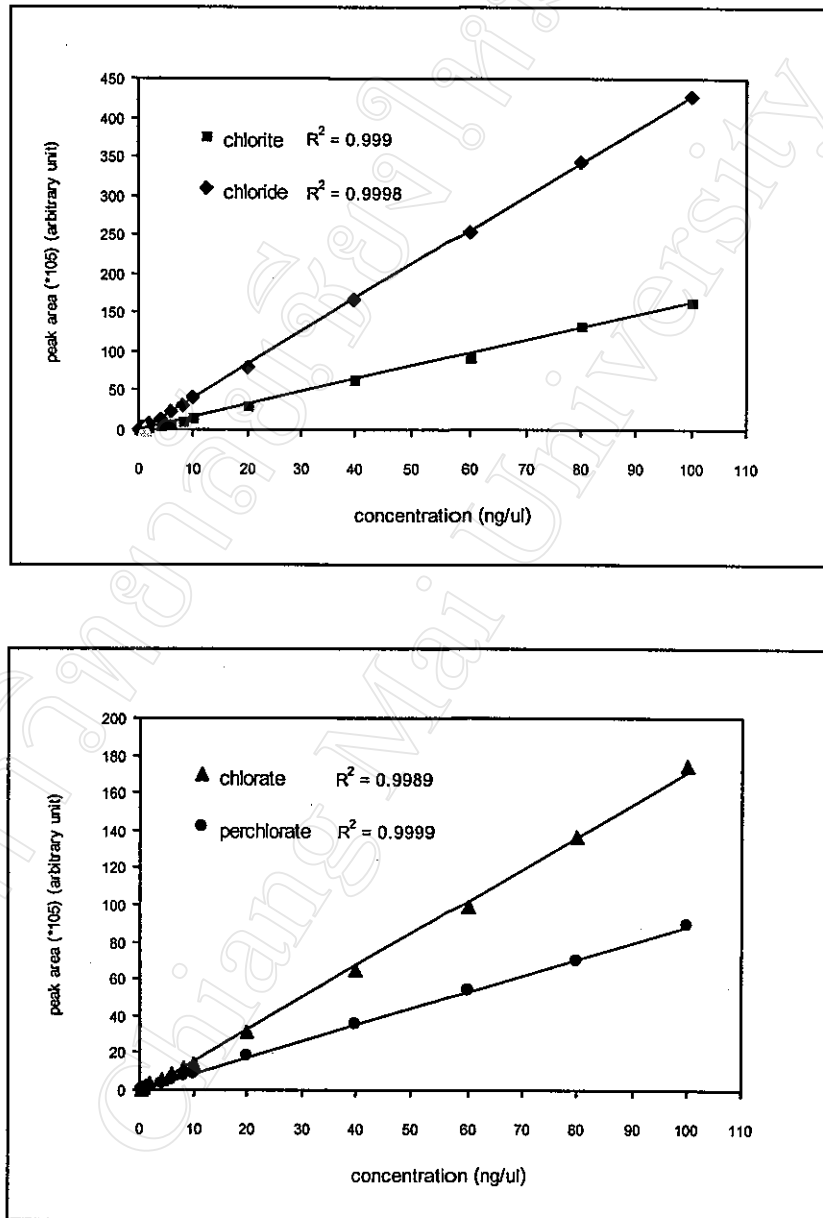


Figure 3.21 The calibration curves for chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.

### 3.4.2 Reproducibility of isocratic system

The seven replicates of chlorine-containing anions solution were introduced onto a Metrosep A Supp 4 column under the established condition. The relative standard deviation values of retention time and peak area of each chlorine-containing anion are shown in Tables 3.25 and 3.26, respectively.

**Table 3.25** Reproducibility of retention time (n=3) of chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min

Run	Average retention time (min)			
	ClO <sub>2</sub> <sup>-</sup> (10.0 ng/μl)	Cl <sup>-</sup> (1.0 ng/μl)	ClO <sub>3</sub> <sup>-</sup> (10.0 ng/μl)	ClO <sub>4</sub> <sup>-</sup> (10.0 ng/μl)
1	2.65	2.98	4.05	19.78
2	2.65	2.98	4.05	19.68
3	2.68	3.02	4.08	19.72
4	2.68	3.02	4.08	19.68
5	2.67	3.00	4.07	19.67
6	2.67	3.00	4.07	19.72
7	2.67	3.00	4.07	19.82
mean	2.67	3.00	4.07	19.72
SD	0.01	0.03	0.01	0.13
%RSD	0.37	1.00	0.25	0.66

**Table 3.26** Reproducibility of peak area (n=3) of chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min

Run	Average peak area (*10 <sup>5</sup> ) (arbitrary unit)			
	ClO <sub>2</sub> <sup>-</sup> (10.0 ng/μl)	Cl <sup>-</sup> (1.0 ng/μl)	ClO <sub>3</sub> <sup>-</sup> (10.0 ng/μl)	ClO <sub>4</sub> <sup>-</sup> (10.0 ng/μl)
1	14.14	3.87	14.30	9.06
2	13.91	3.79	14.41	8.87
3	14.07	3.80	14.42	8.92
4	14.15	3.65	14.47	8.95
5	14.18	3.68	14.38	8.75
6	14.14	3.68	14.38	8.98
7	14.61	3.67	14.24	9.02
mean	14.17	3.73	14.37	8.94
SD	0.28	0.08	0.08	0.10
%RSD	1.98	2.14	0.56	1.12

### 3.4.3 Detection limit of isocratic system

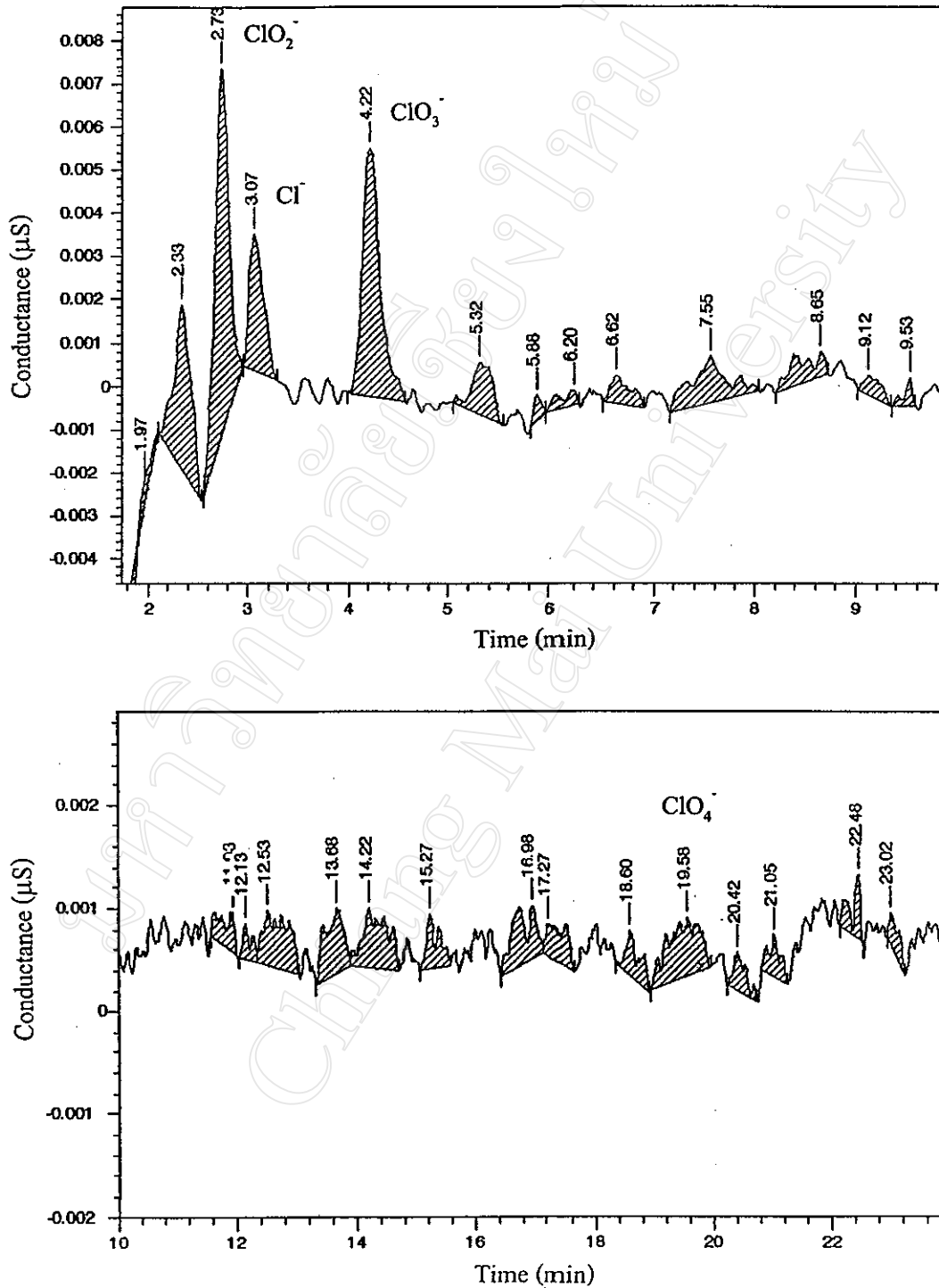
The lowest concentration chlorine-containing anions, chlorite, chloride, chlorate and perchlorate was used for determination of detection limit. The obtained results and the chromatograms of chlorine-containing anions are shown in Tables 3.27-3.28 and Figure 3.22, respectively.

**Table 3.27** The data of noise level (n), peak signal response (R) and width at half height of peak ( $w_{0.5}$ ) of chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min

Data	Response of chlorine-containing anions			
	$\text{ClO}_2^-$	$\text{Cl}^-$	$\text{ClO}_3^-$	$\text{ClO}_4^-$
Height of noise level ( $\mu\text{S}$ )	0.0002	0.0002	0.0003	0.0004
	0.0003	0.0003	0.0006	0.0003
	0.0003	0.0005	0.0005	0.0005
mean	0.0002	0.0003	0.0004	0.0004
Height of peak signal response ( $\mu\text{S}$ )	0.0067	0.0097	0.0056	0.0006
	0.0063	0.0174	0.0060	0.0005
	0.0056	0.0117	0.0058	0.0006
	0.0080	0.0032	0.0059	0.0007
	0.0060	0.0264	0.0058	0.0007
	0.0056	0.0265	0.0058	0.0005
	0.0070	0.0209	0.0060	0.0004
Mean	0.0065	0.0165	0.0058	0.0006
Width at half height of peak (sec)	0.127	0.126	0.170	0.268
	0.120	0.097	0.195	0.169
	0.117	0.115	0.179	0.586
	0.113	0.169	0.187	0.139
	0.131	0.124	0.176	0.260
	0.116	0.135	0.191	0.254
	0.131	0.135	0.191	0.172
Mean	0.122	0.129	0.184	0.264

**Table 3.28** The detection limit (L) and minimum detectable quantity (MDQ) of chlorine-containing anions obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min

Chlorine-containing anions	Concentration (ng/ $\mu$ l)	Detection limit (L)(ng)	Minimum detectable quantity (MDQ)( $\times 10^{-2}$ )(ng.sec)
$\text{ClO}_2^-$	0.05	0.005	0.06
$\text{Cl}^-$	0.05	0.002	0.02
$\text{ClO}_3^-$	0.05	0.007	0.13
$\text{ClO}_4^-$	0.05	0.07	1.85



**Figure 3.22** Chromatograms of chlorine-containing anions at lowest concentration, are shown the peak signal response ( $R$ ) and width at half height of peak ( $w_{0.5}$ ) using with a Metrosep A Supp 4 column and a conductivity detector with 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.

### 3.4.4 Recovery of chlorine-containing anions in the water samples using isocratic system

The accuracy of quantification of chlorine-containing anions with the Metrosep A Supp 4 column was improved by the spiked recovery method. The water samples were selected for this investigation consisting of (1) Maharaj Nakorn Chiang Mai Hospital, (2) Rujirawong swimming pool in Chiang Mai University and (3) Mae-Ping river. The obtained results of %recoveries of each chlorine-containing anion are shown in Tables 3.29-3.32 and the chromatograms of the spiked water samples and non-spiked water samples are presented in Figures 3.23 and 24.

**Table 3.29** The percentage recovery of chlorite in the water samples with the spiked recovery method at various concentrations using isocratic system

Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	2.0	219810	1.89	99.50
	4.0	470540	3.43	85.75
	8.0	1011970	6.75	84.38
Rujirawong swimming pool water (Sample No.11)	2.0	236513	1.99	99.50
	4.0	498217	3.59	89.75
	8.0	1076623	7.15	89.38
Natural water in Mae-Ping river (Sample No.15)	2.0	200670	1.77	88.50
	4.0	517083	3.71	92.75
	8.0	1038237	6.91	86.38
Recovery range		84.38-99.50% (mean 90.07%)		
SD		4.79		
%RSD		5.32%		

**Table 3.30** The percentage recovery of chloride in the water samples with the spiked recovery method at various concentrations using isocratic system

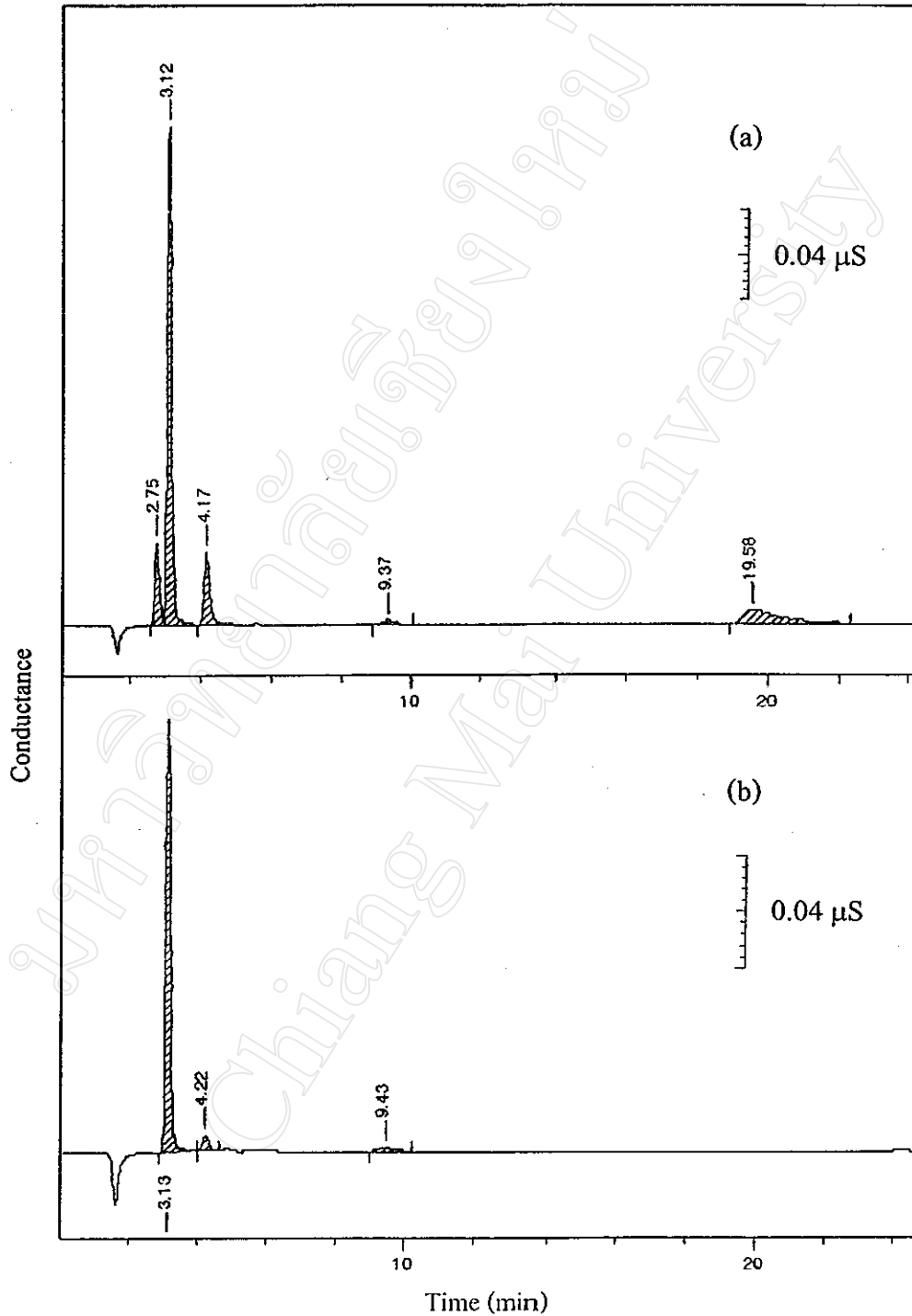
Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	1.0	4277275	1.19	119.00
	2.0	4709947	2.23	111.50
	4.0	5735170	4.70	117.50
Rujirawong swimming pool water (Sample No.11)	1.0	2616193	1.16	116.00
	2.0	3088787	2.30	115.00
	4.0	4117130	4.78	119.50
Natural water in Mae-Ping river (Sample No.15)	1.0	1828820	1.03	103.00
	2.0	2410053	2.43	121.50
	4.0	3333470	4.65	116.25
Recovery range	103.00-121.50% (mean 115.47%)			
SD	5.49			
%RSD	4.75%			

**Table 3.31** The percentage recovery of chlorate in the water samples with the spiked recovery method at various concentrations using isocratic system

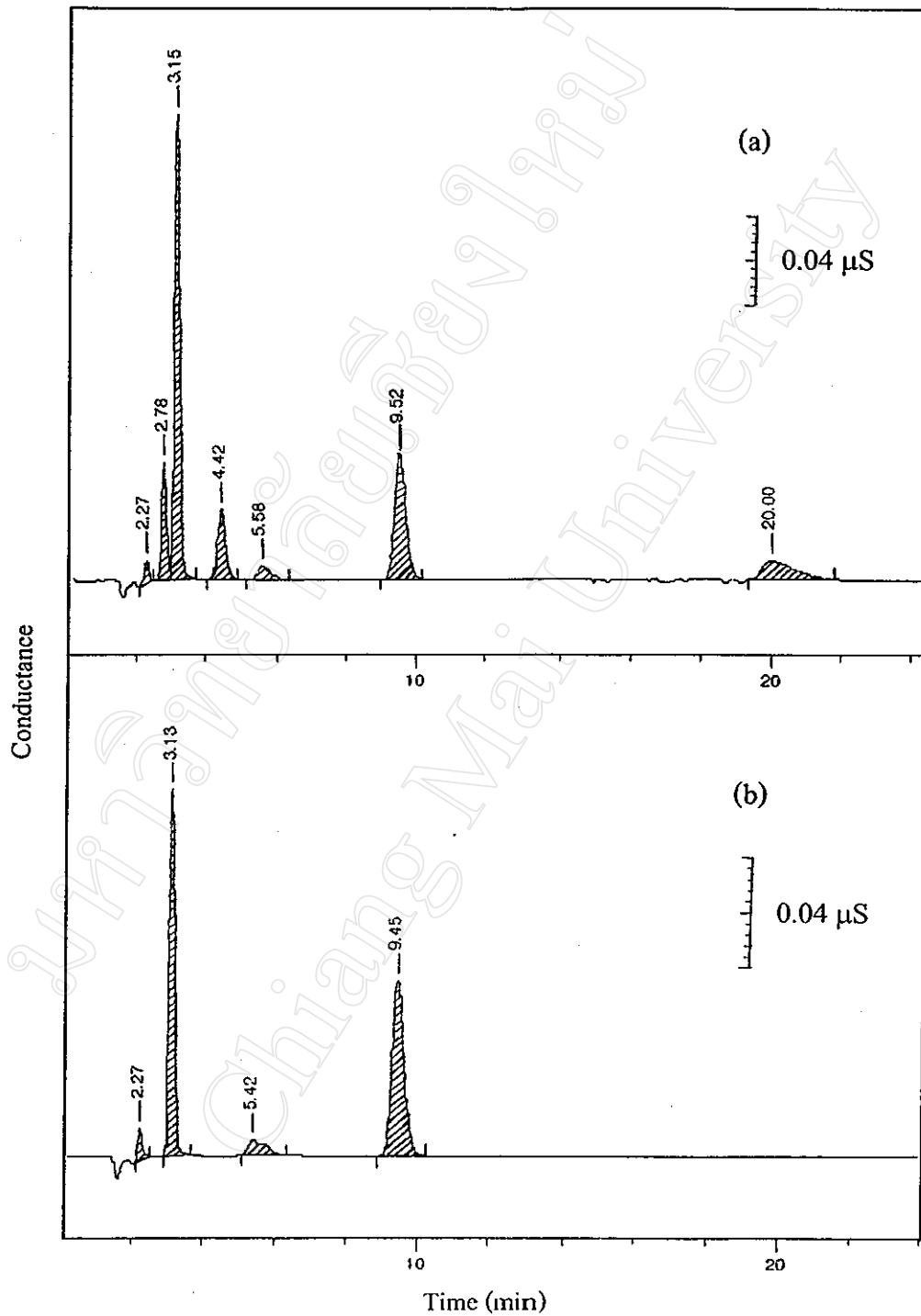
Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	2.0	343097	2.46	80.00
	4.0	615320	4.10	81.00
	8.0	1204847	7.65	84.88
Rujirawong swimming pool water (Sample No.11)	2.0	355853	2.54	81.50
	4.0	635640	4.22	82.75
	8.0	1250487	7.92	87.63
Natural water in Mae-Ping river (Sample No.15)	2.0	223490	1.74	87.00
	4.0	520920	3.41	85.25
	8.0	1092223	6.79	87.13
Recovery range	80.00-87.63% (mean 84.13%)			
SD	2.89			
%RSD	3.44%			

**Table 3.32** The percentage recovery of perchlorate in the water samples with the spiked recovery method at various concentrations using isocratic system

Sample	Concentration spiked (ng/ $\mu$ l)	Average peak area (arbitrary unit)	Concentration found (ng/ $\mu$ l)	%recovery
Public water supply in Maharaj Nakorn Chiang Mai Hospital (Sample No.10)	4.0	358933	3.90	97.50
	8.0	745673	7.94	99.25
	16.0	1496120	15.78	98.63
Rujirawong swimming pool water (Sample No.11)	4.0	361757	3.94	98.50
	8.0	752133	8.01	100.13
	16.0	1493837	15.76	98.50
Natural water in Mae-Ping river (Sample No.15)	2.0	361680	3.94	98.50
	4.0	743760	7.93	99.13
	8.0	1479433	15.60	97.50
Recovery range		97.50-100.13% (mean 98.63%)		
SD		0.73		
%RSD		0.74%		



**Figure 3.23** The chromatograms of (a) the spiked and (b) non-spiked samples of Rujirawong swimming pool water (50 fold dilution) in Chiang Mai University obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.

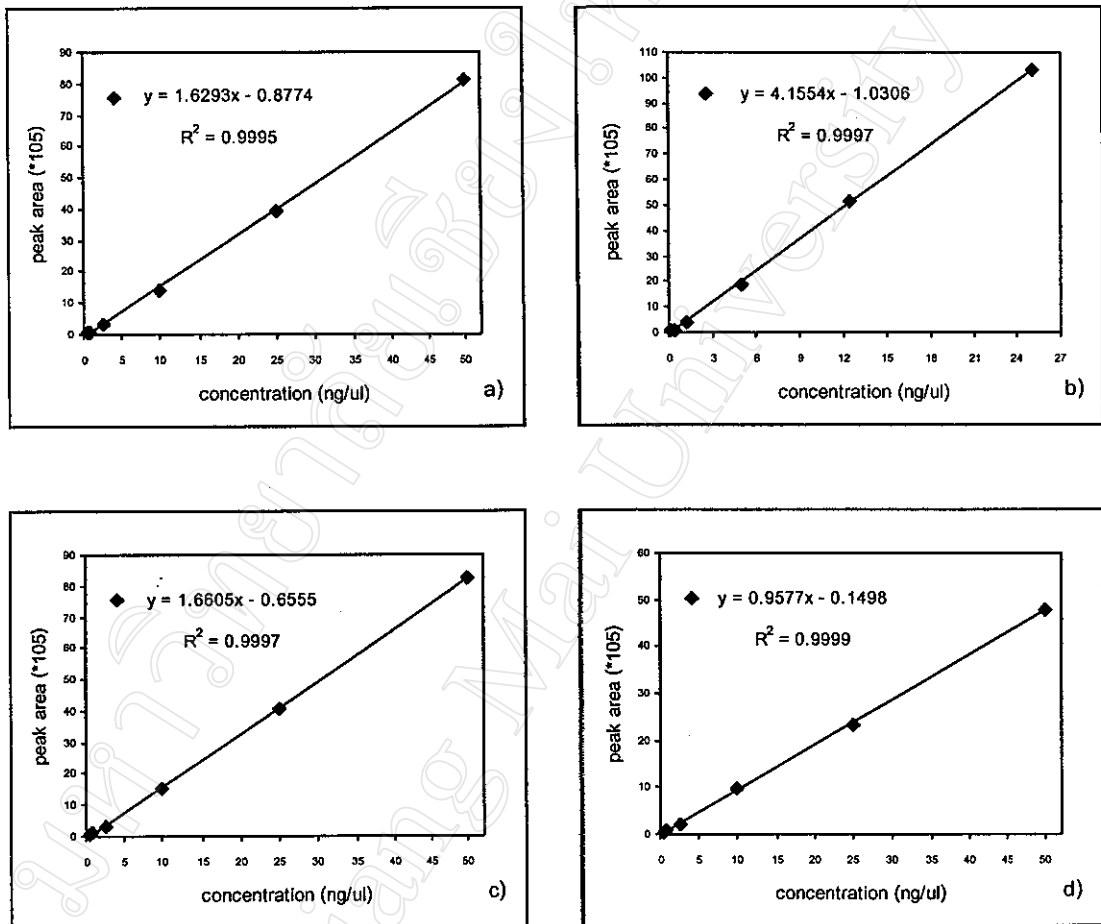


**Figure 3.24** The chromatograms of (a) the spiked and (b) non-spiked samples of natural water in Mae-Ping river obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.

### 3.4.5 Determination of chlorine-containing anions in the water samples using isocratic system

The external standard method was used in the investigation by the calibration curves of chlorine-containing anions, constructed from average peak areas and concentrations of chlorite, chlorate and perchlorate in the range of 0.4-50.0 ng/ $\mu$ l and chloride in the range of 0.2-25.0 ng/ $\mu$ l, as shown in Figure 3.25.

The determination of chlorine-containing anions in the water samples was carried out using the isocratic system obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min. From the analysis, the chlorine-containing anions, which were found in the water samples, were chloride and chlorate. The results are shown in Table 3.33 and the chromatograms of some water samples are presented in Figures 3.26-3.29.



**Figure 3.25** The calibration curves of chlorine-containing anions

a) chlorite, b) chloride, c) chlorate and d) perchlorate.

**Table 3.33** The results of determination of chlorine-containing anions in the water samples obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min

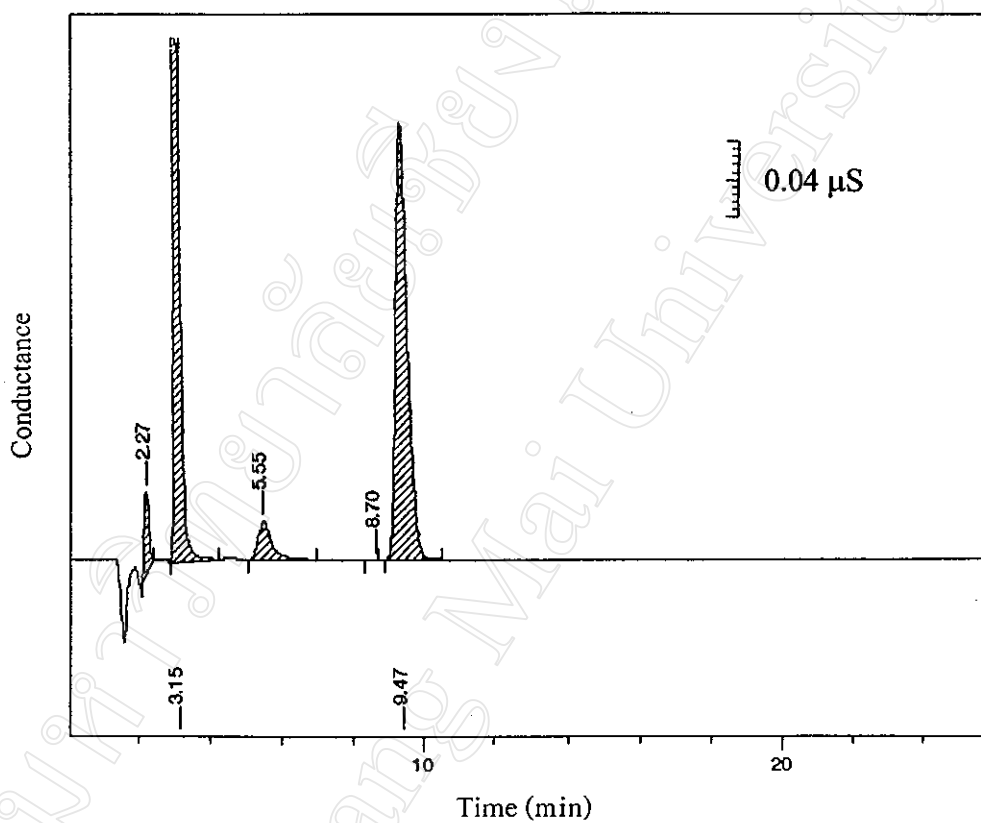
Sample No.	Chloride ion		Chlorate ion	
	Average peak area	Concentration (ng/ $\mu$ l)	Average peak area	Concentration (ng/ $\mu$ l)
Sample No.1	1956096	4.96	-	-
Sample No.2	2508320	6.28	-	-
Sample No.3	2360973	5.23	-	-
Sample No.4	1963937	4.97	-	-
Sample No.5	1988486	5.03	-	-
Sample No.6	1614299	4.13	-	-
Sample No.7	1952465	4.95	-	-
Sample No.8	4899507	12.04	-	-
Sample No.9	7736533	18.87	-	-
Sample No.10	3781085	9.35	78380	0.86
Sample No.11 <sup>a</sup>	2134233	5.38 (269.00 <sup>d</sup> )	85970	0.91 (45.50 <sup>d</sup> )
Sample No.12 <sup>b</sup>	5280765	12.96 (324.00 <sup>d</sup> )	80583	0.88 (22.00 <sup>d</sup> )
Sample No.13 <sup>c</sup>	8846547	21.54 (430.80 <sup>d</sup> )	81060	0.88 (17.60 <sup>d</sup> )
Sample No.14	1077042	2.84	-	-
Sample No.15	1400050	3.62	-	-

<sup>a</sup> 50 fold dilution

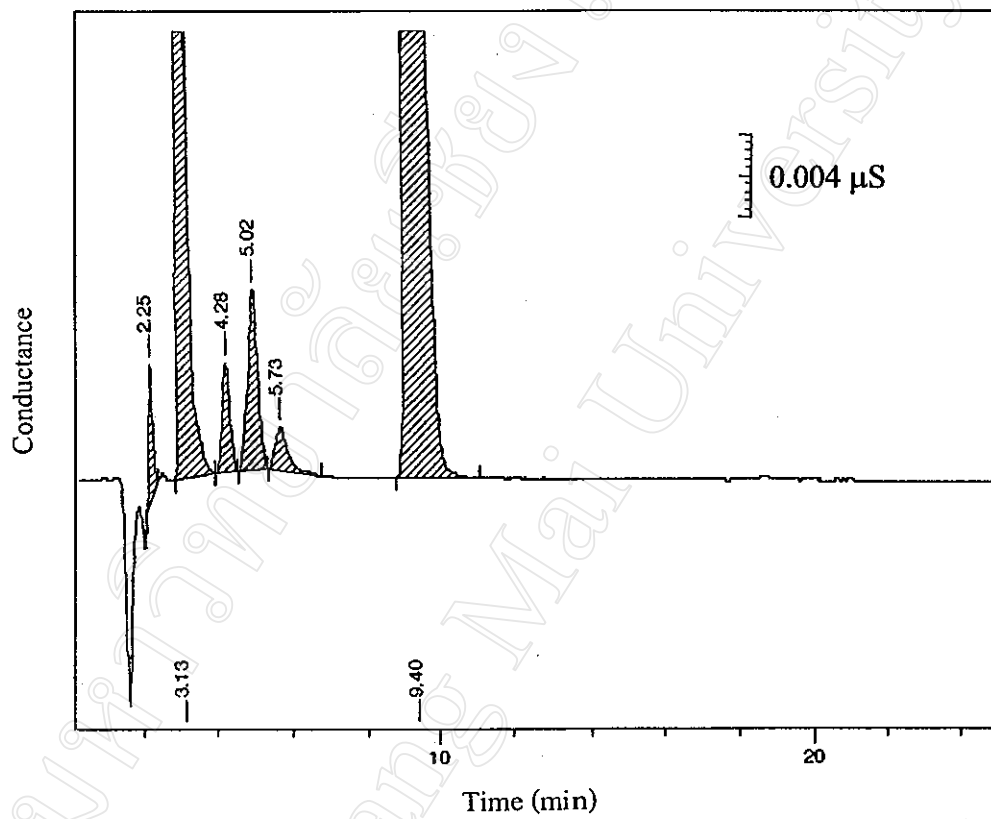
<sup>c</sup> 20 fold dilution

<sup>b</sup> 25 fold dilution

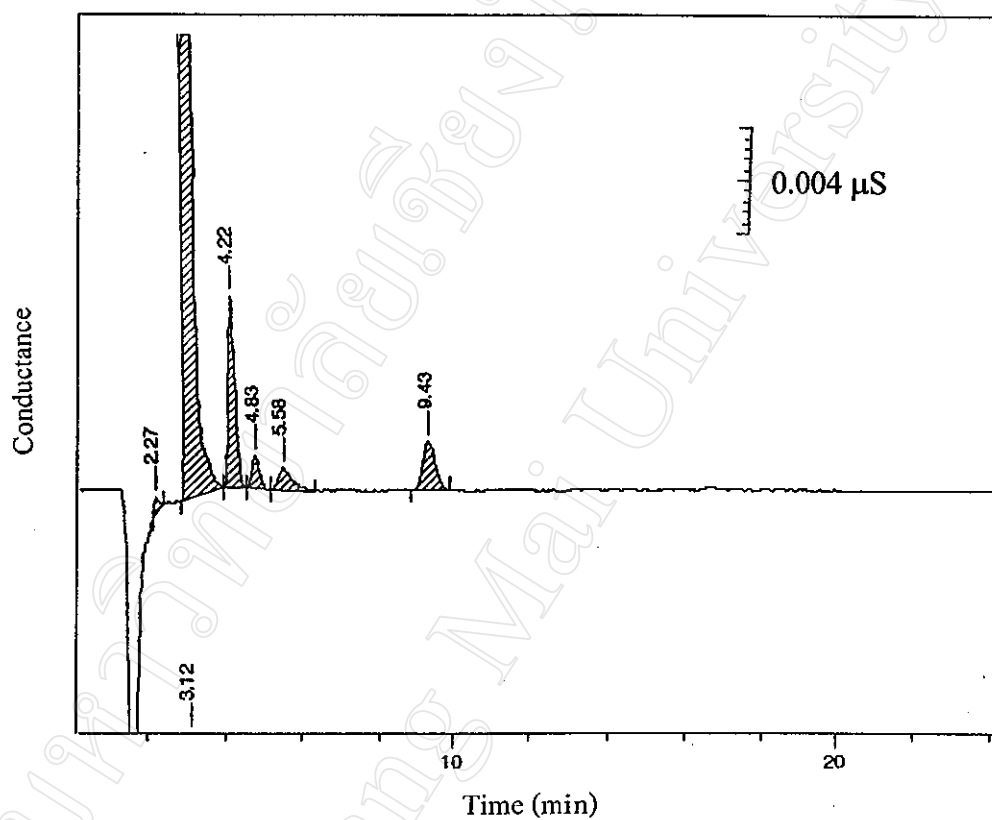
<sup>d</sup> original concentration



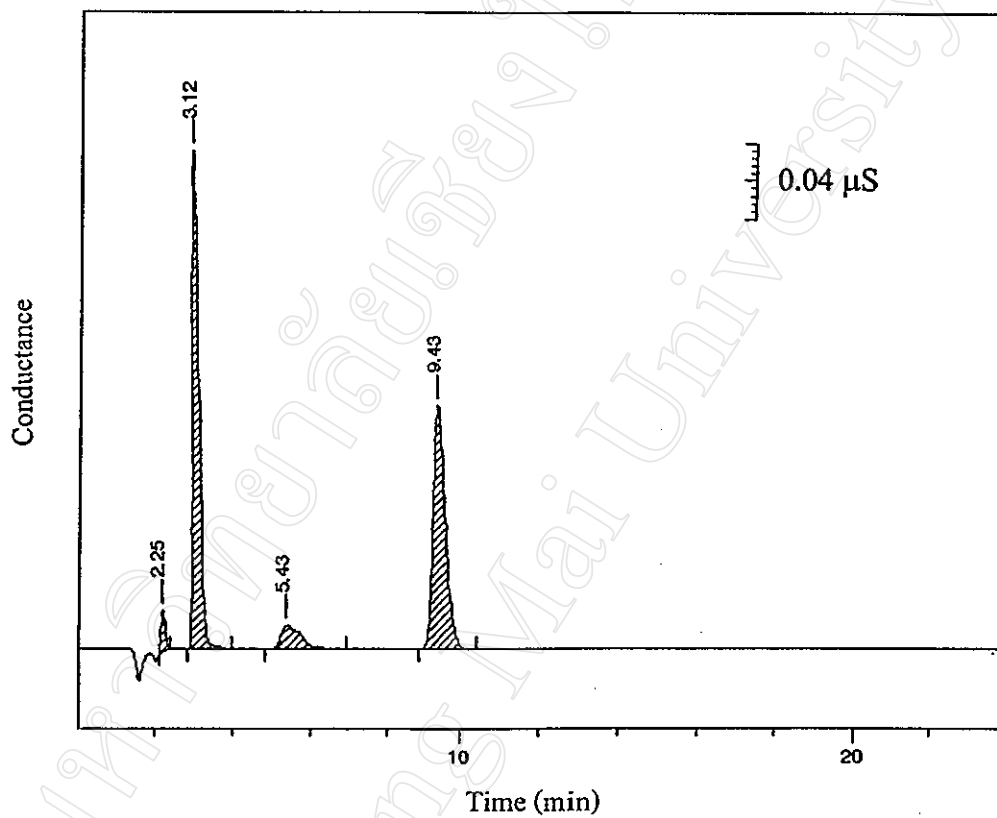
**Figure 3.26** The chromatogram of the natural water in longan orchard (sample No.6) obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.



**Figure 3.27** The chromatogram of the public water supply in Maharaj Nakorn Chiang Mai Hospital (sample No.10) obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.



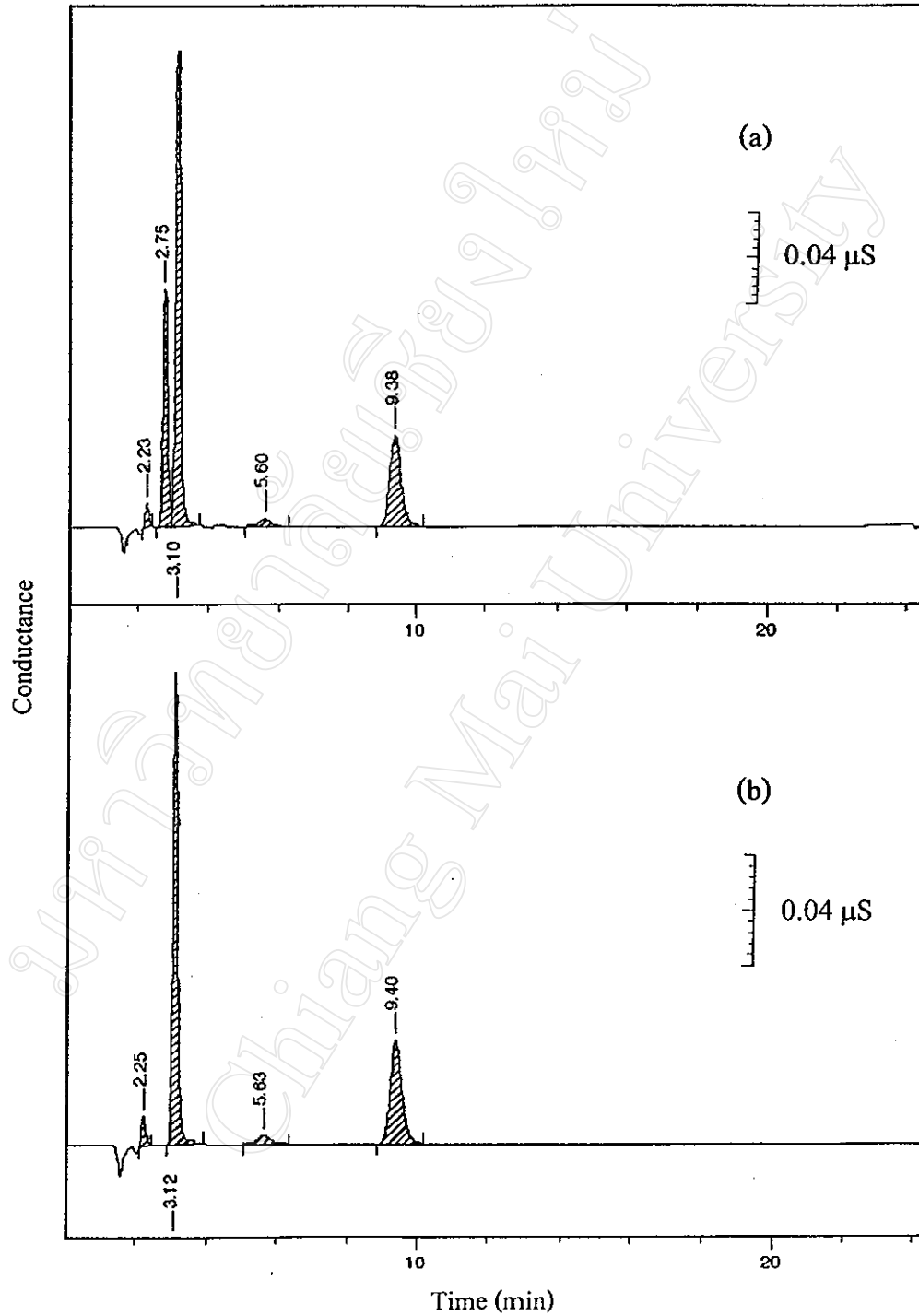
**Figure 3.28** The chromatogram of Rujirawong swimming pool water in Chiang Mai University (sample No.11, 50 fold dilution) obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.



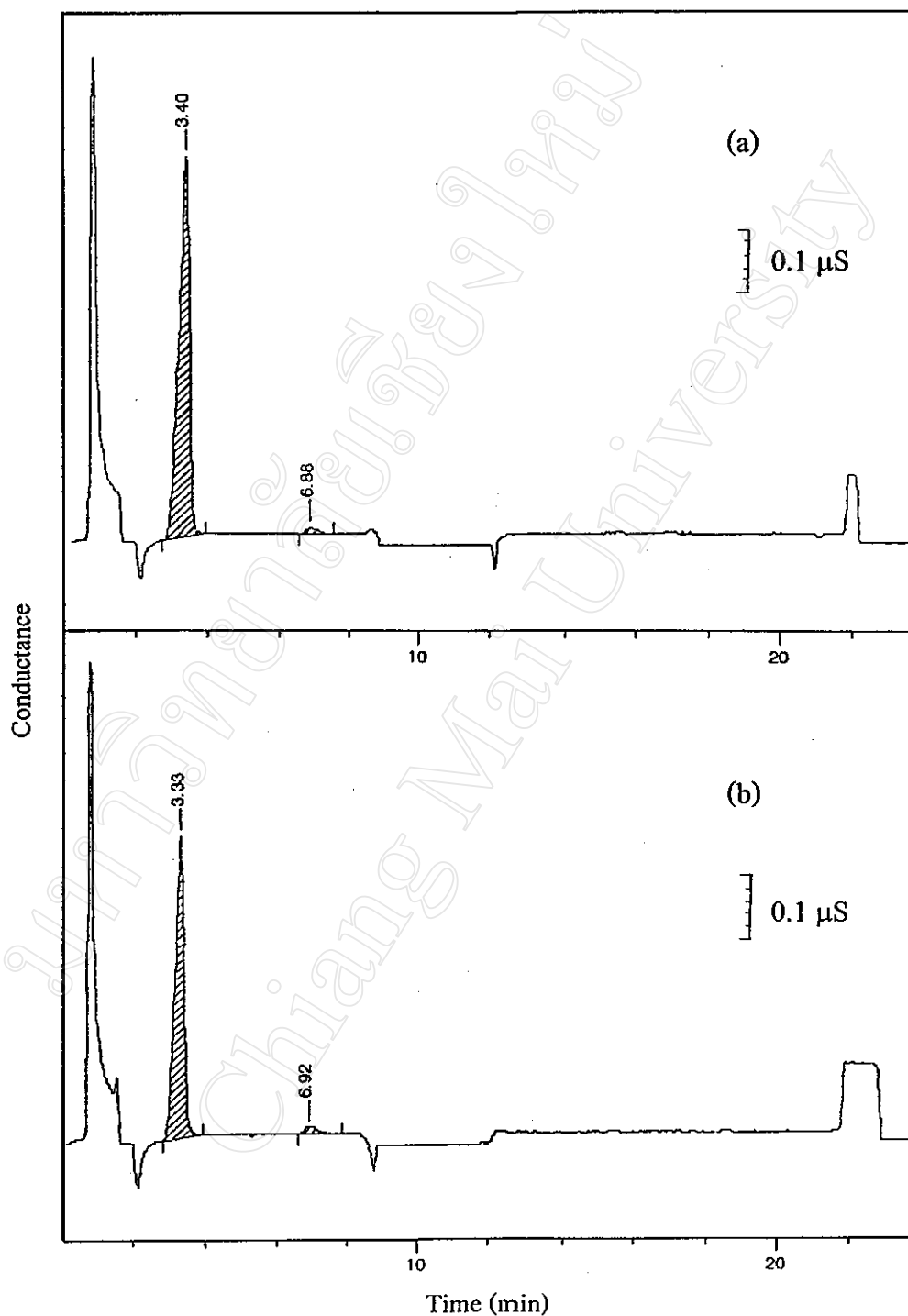
**Figure 3.29** The chromatogram of the natural water in Mae-Ping river (sample No.15) obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.

### 3.5 Identification of Chlorine-containing Anions in the Water Samples

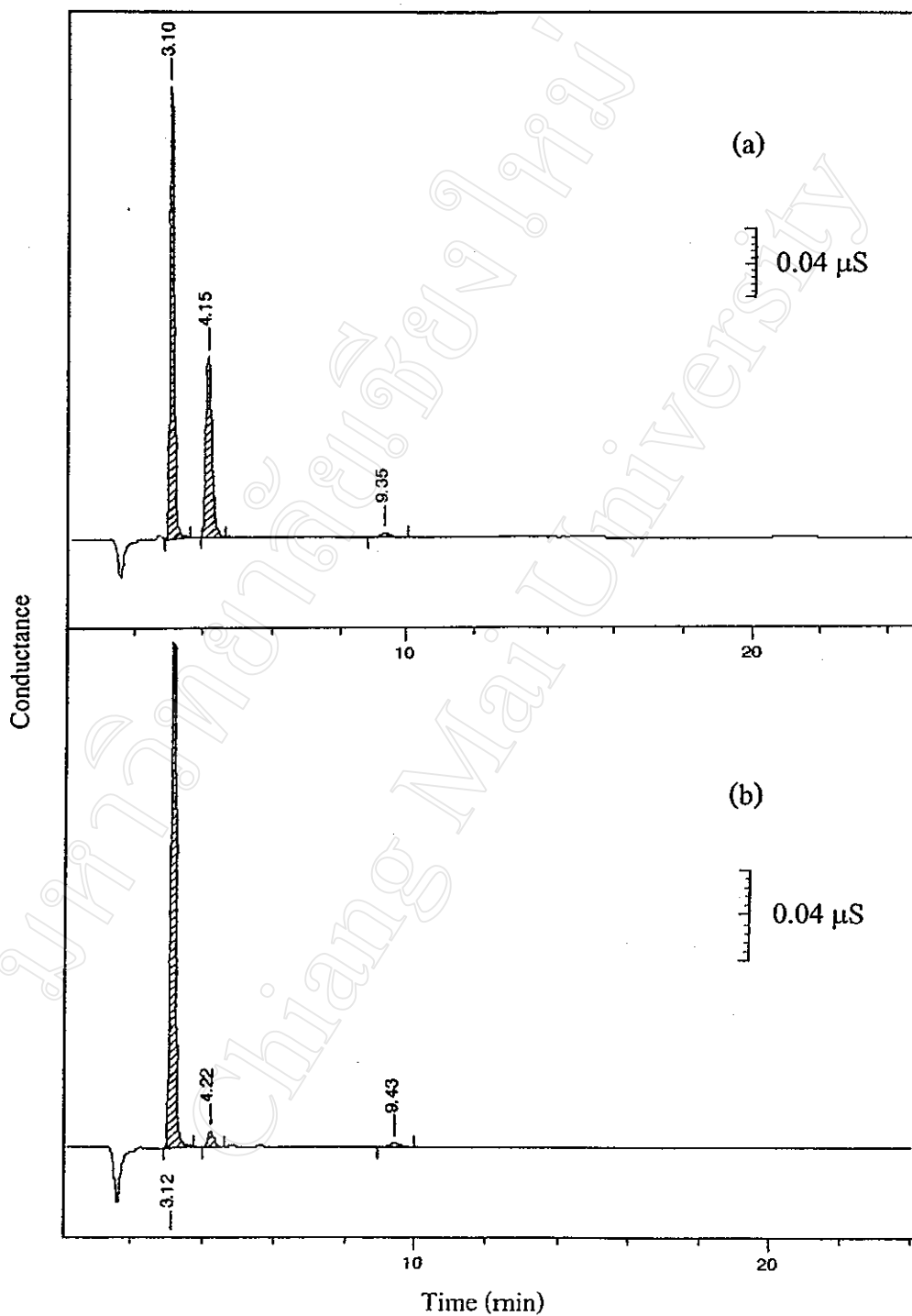
Identification of chlorine-containing anions found in the water samples, the addition method was employed in this work by adding the individual standard anions into the samples. The comparison between the chromatograms of the water samples and the added chlorite, chloride, chlorate and perchlorate samples are presented in Figures 3.30-3.33, respectively.



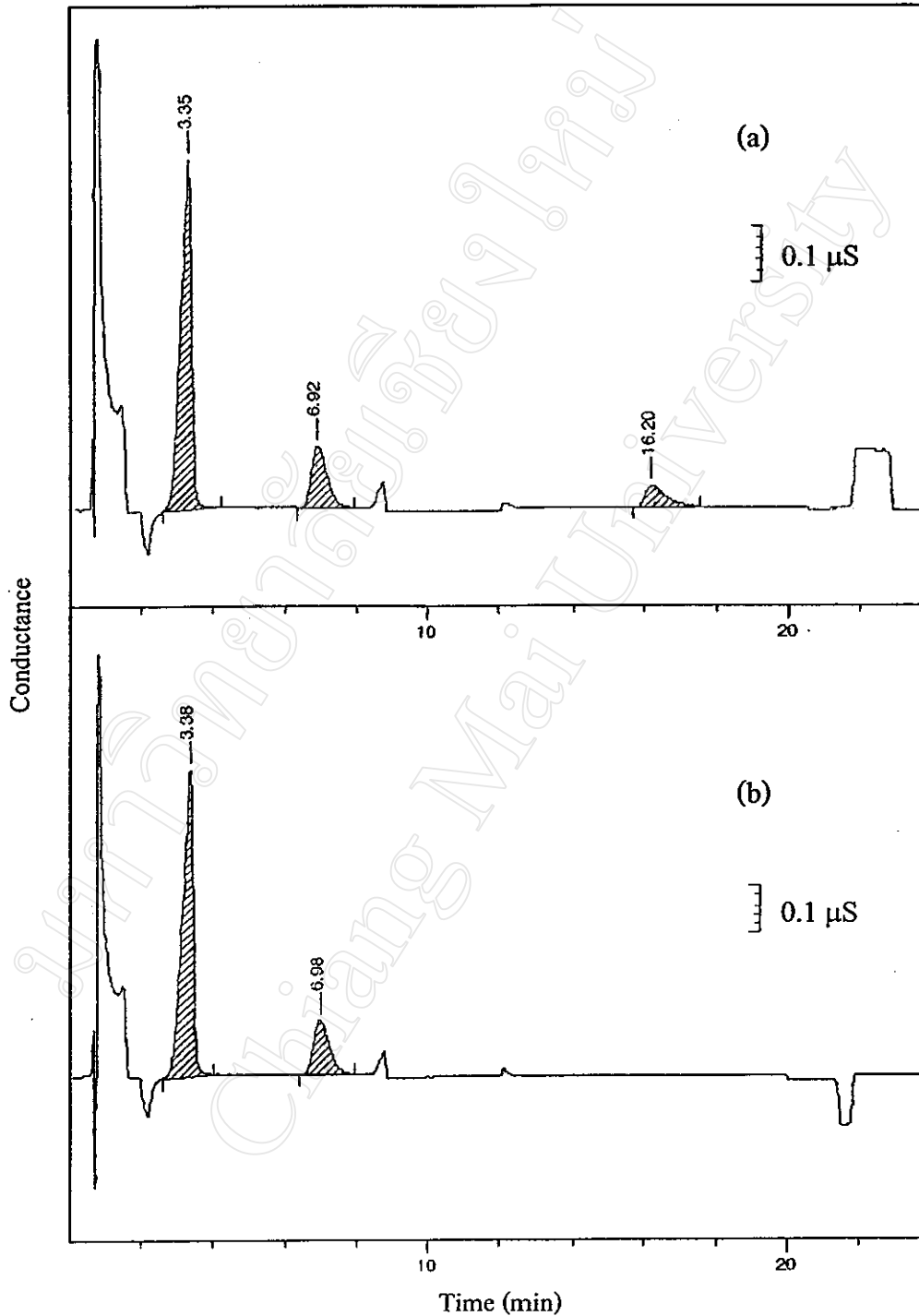
**Figure 3.30** Comparison of the chromatograms of (a) the added and (b) non-added chlorite samples of natural water in longan orchard (sample No.7) obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.



**Figure 3.31** Comparison of the chromatograms of (a) the added and (b) non-added chloride samples of swimming pool water in the 103 Condominium (sample No.12, 25 fold dilution) obtained with an IonPac AS4A-SC column and gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.



**Figure 3.32** Comparison of the chromatograms of (a) the added and (b) non-added chlorate samples of Rujirawong swimming pool water (sample No.11, 50 fold dilution) obtained with a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min.



**Figure 3.33** Comparison of the chromatograms of (a) the added and (b) non-added perchlorate samples of public water supply in Providence Hotel (sample No.9) obtained with an IonPac AS4A-SC column and gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min.

### 3.6 Comparison of the Quantitative Analysis of the Water Samples Using Gradient and Isocratic Systems

The comparison of the analysis of chlorine-containing anions in the water samples between gradient and isocratic systems using the F test and the t test. The results obtained with both systems are shown in Tables 3.34-3.37.

**Table 3.34** The results of the F test between the comparison of concentration of chloride ion in the water samples using gradient and isocratic systems

Sample No.	Gradient system			Isocratic system		
	1 <sup>st</sup> time	2 <sup>nd</sup> time	3 <sup>rd</sup> time	1 <sup>st</sup> time	2 <sup>nd</sup> time	3 <sup>rd</sup> time
Sample No.1	4.76	4.70	5.03	4.87	5.10	4.91
Sample No.5	5.27	4.97	4.96	5.00	5.14	4.95
Sample No.8	11.54	11.68	11.75	12.10	11.87	12.15
Sample No.10	9.19	9.23	9.00	9.45	9.39	9.21
Sample No.15	3.71	3.77	3.64	3.55	3.66	3.65
SD <sup>2</sup>	0.0186			0.0133		
F <sub>cal</sub> = 1.40 (F <sub>table</sub> = 2.98, 95% confidence level at V <sub>1</sub> =10, V <sub>2</sub> =10)						

**Table 3.35** The results of the F test between the comparison of concentration of chlorate ion in the water samples using gradient and isocratic systems

Sample No.	Gradient system			Isocratic system		
	1 <sup>st</sup> time	2 <sup>nd</sup> time	3 <sup>rd</sup> time	1 <sup>st</sup> time	2 <sup>nd</sup> time	3 <sup>rd</sup> time
Sample No.10	0.81	0.92	0.93	0.92	0.84	0.82
Sample No.12	1.02	0.88	0.90	0.86	0.89	0.89
Sample No.13	0.85	0.89	0.99	0.87	0.89	-
SD <sup>2</sup>	0.0054			0.0008		
$F_{cal} = 6.75$ ( $F_{table} = 4.95$ , 95% confidence level at $V_1=6$ , $V_2=5$ )						

**Table 3.36** The results of the t test between the comparison of concentration of chloride ion in the water samples using gradient and isocratic systems

Sample No.	Gradient system	Isocratic system	$D_i$	$(D_i - \bar{D})^2$
Sample No.1	4.83	4.96	0.13	0.0016
Sample No.5	5.07	5.03	0.39	0.0484
Sample No.8	11.65	12.04	0.20	0.0009
Sample No.10	9.15	9.35	0.04	0.0169
Sample No.15	3.70	3.62	0.08	0.0081
Mean of deviation ( $\bar{D}$ ) = 0.17 Standard deviation (SD) = 0.14 $t_{cal} = 2.72$ ( $t_{table} = 2.78$ at 95% confidence level)				

**Table 3.37** The results of the t test between the comparison of concentration of chlorate ion in the water samples using gradient and isocratic systems

Sample No.	Gradient system	Isocratic system	$D_i$	$(D_i - \bar{D})^2$
Sample No.10	0.89	0.86	0.03	0.0676
Sample No.12	9.33	8.76	0.57	0.0784
Sample No.13	9.10	8.83	0.27	0.0004
Mean of deviation ( $\bar{D}$ ) = 0.29 Standard deviation (SD) = 0.27 $t_{cal} = 2.15$ ( $t_{table} = 4.30$ at 95% confidence level)				

From the analysis of performance and accuracy of the gradient and isocratic systems, the obtained results were directed to compare, which can be summarized in Table 3.38, in the following:

**Table 3.38** Comparison of linearity, detection limit, reproducibility and recovery for analysis of chlorine-containing anions using gradient and isocratic systems

Chlorine-containing anion	The linearity range (ng/ $\mu$ l) ( $r^2$ )		Detection limit (ng)		
	GS	IS	GS	IS	
$\text{ClO}_2^-$	0.4-200 (0.9890)	0.1-100 (0.9999)	0.03	0.005	
$\text{Cl}^-$	0.2-100 (0.9998)	0.1-100 (0.9998)	0.01	0.002	
$\text{ClO}_3^-$	0.2-200 (0.9999)	0.1-100 (0.9989)	0.02	0.007	
$\text{ClO}_4^-$	0.4-200 (0.9999)	0.2-100 (0.9999)	0.06	0.07	
Chlorine-containing anion	Reproducibility of $t_r$ , %RSD		Reproducibility of PA, %RSD		%recovery (%RSD)
	GS	IS	GS	IS	
$\text{ClO}_2^-$	0.43	0.37	3.68	1.98	85.40 (4.41%) 90.07 (5.32%)
$\text{Cl}^-$	0.61	1.00	3.44	2.14	112.97 (5.46%) 115.47 (4.75%)
$\text{ClO}_3^-$	0.30	0.25	1.33	0.56	85.91 (3.06%) 84.13 (3.44%)
$\text{ClO}_4^-$	0.30	0.66	1.21	1.12	99.27 (1.05%) 98.63 (0.74%)

GS : gradient system

IS : isocratic system