# CHAPTER 3

RESULTS AND DISCUSSION

3.1 Analytical characteristics of the method

3.1.1 Linearity

The linearity of the method was examined in the concentration range of 10 to 120 ppb within triplicate performance of each concentration. The linearity range obtained from the experimental calibration curves is presented in **Table 3.1** and calibration curves are shown in **Figure 3.1**. This indicated that the linear range was in the range of 10 to 100 ppb which was useful for analysis of total Fe, extracted Fe, Fe^{2+} and Fe^{3+} in rice samples.

Table 3.1 Linear range of standard Fe calibration curve

Fe concentration (ppb)	Abs.
10	0.0437
20	0.0978
30	0.1434
40	0.1826
50	0.2281
60	0.2623
70	0.3012
80	0.3509
90	0.3821
100	0.4185
110	0.4301
120	0.4415
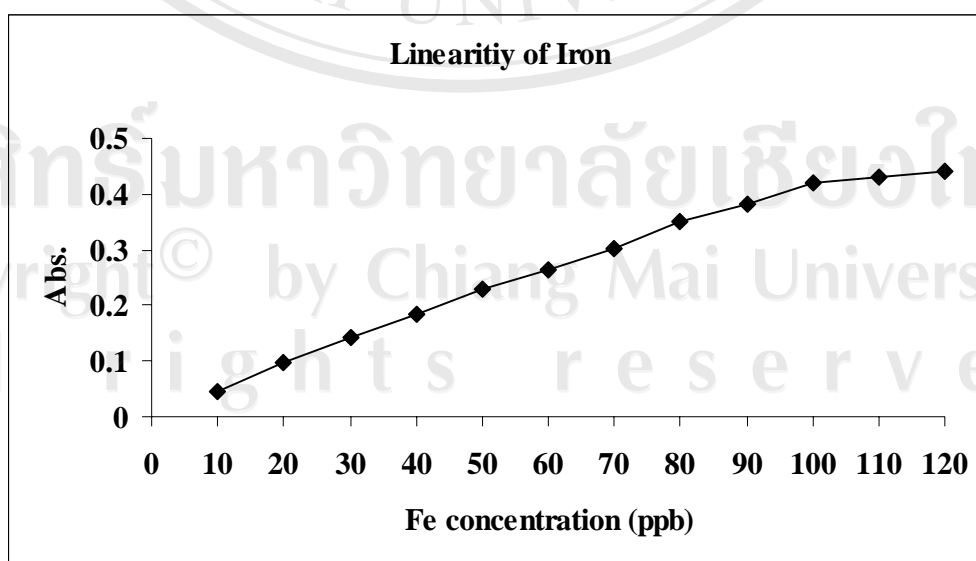
Figure 3.1 Linearity plot of absorbance against concentration of iron.

3.1.2 Precision

The precision of the GFAAS instrument was studied by repeating the measurement of 20 ppb standard solutions ten times. The results obtained are shown in **Tables 3.2**. The results exhibited that the precision with replicate injections, expressed as the relative standard deviation (%RSD), was found to be 1.65%. This indicated that the GFAAS instrument provided good repeatability (less than 5 %RSD) for iron under the optimum conditions.

Tables 3.2 The precision of the GFAAS instrument for Fe analysis

No	Abs. of Fe 20 ppb
1	0.0987
2	0.0975
3	0.0956
4	0.1002
5	0.0970
6	0.0972
7	0.0969
8	0.0981
9	0.0975
10	0.1010
Mean	0.0980
SD	0.0016
%RSD	1.65

3.1.3 Detection limit

The detection limit of the method for determination of iron was investigated by analyzing blank solution of 0.2% HNO_3 ten times. The results are given in **Table 3.3**. The detection limit was found to be 0.29 ppb. The calculation of detection limit value is shown in Appendix C.

Table 3.3 Absorbance obtained from blank solution

No.	Abs. of 0.2%HNO_3
1	0.0181
2	0.0175
3	0.0180
4	0.0179
5	0.0176
6	0.0174
7	0.0169
8	0.0182
9	0.0175
10	0.0179
Mean	0.0177
SD	0.0004
Slope	0.0041
Detection limit (ppb)	0.2882

3.1.4 Accuracy

The method was examined by determining the recoveries of the added 60 ppb of Fe^{2+} and Fe^{3+} in sample solution. The results are presented in **Table 3.4**. The average recoveries were found in the range of 76.3-115%. The proposed method for speciation of Fe^{2+} and Fe^{3+} was found to be accurate for the determination of Fe^{2+} and Fe^{3+} in sample solutions. The calculation of accuracy is shown in Appendix C.

Table 3.4 The average recoveries of Fe^{2+} analysis

| Sample	Concentration of Fe^{2+} (μg/kg)		%Recovery
	Added	Found	
Rice bran-1	-	24.8	
	60	93.1	114
Rice bran-2	-	89.8	
	60	139.8	83.4
Rice bran-3	-	66.0	
	60	134.9	115
Rice bran-4	-	70.8	
	60	138.5	113
Rice bran-5	-	95.0	
	60	140.7	76.3

3.2 Determination of total iron in rice samples

A total Fe was determined in the powdered samples by microwave digestion and GFAAS as described in **section 2.5**. **Table 3.5** and **Figure 3.2** show the total Fe content in rice and rice bran samples. Example of the calculation is shown in Appendix C. The results indicated that the amounts of total Fe in brown rice samples are higher than black rice, red rice and rice bran samples, respectively. The amount of total Fe in Rice-3 was the highest of all samples. Thus, Rice-3 was chosen for optimization of extraction method that the experimental procedure was shown in **section 2.6**. The amount of total Fe was little. The amount of extracted Fe was less than total Fe and the amount of Fe^{2+} was the least. Because the amount of Fe^{2+} was obtained from extracted Fe and the amount of extracted Fe was obtained from total Fe, too. So, the rice sample that was the highest of total Fe was selected.

Table 3.5 The total Fe content in rice and rice bran samples

Sample	Fe content* (µg/kg)	%RSD
Rice-1	488.8±45.2	9.2
Rice-2	407.8±43.5	10.7
Rice-3	640.8±32.9	5.1
Rice-4	351.4±36.7	10.4
Rice-5	512.8±17.4	3.4
Rice-6	468.5±6.8	1.5
Rice bran-1	92.00±5.6	6.1
Rice bran-2	160.8±14.4	9.0
Rice bran-3	120.8±23.5	19.5
Rice bran-4	101.5±13.7	13.5
Rice bran-5	138.3±8.1	5.9

*Mean±SD (N=3)
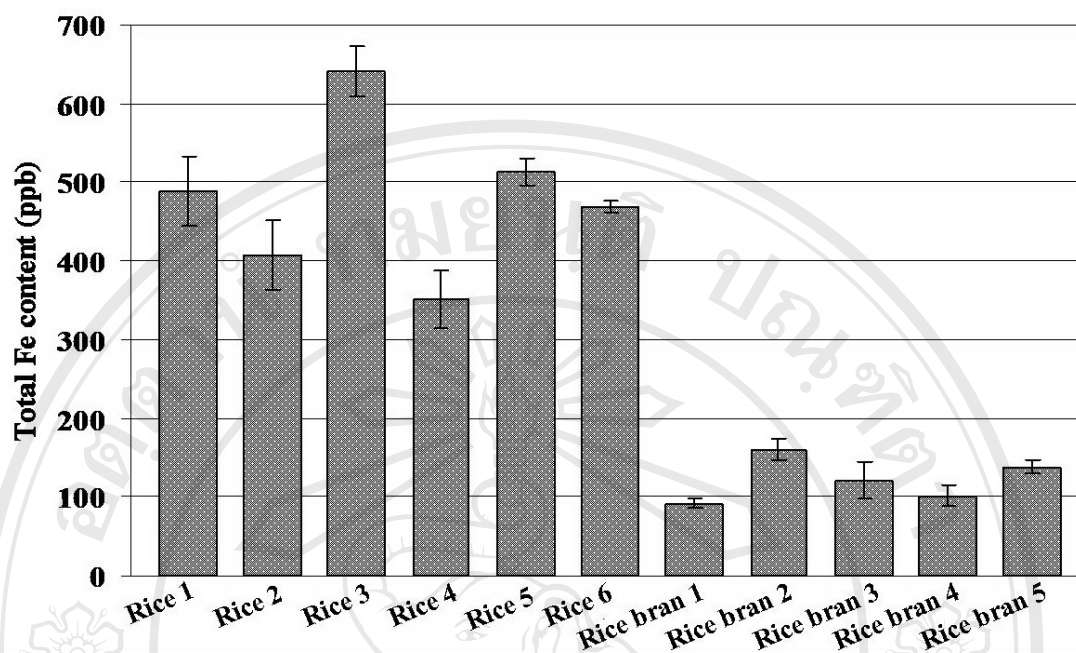
Figure 3.2 The histogram of total Fe content in rice and rice bran samples.

3.3 Optimization of extraction method

Seven kinds of solutions including MIBK, $CHCl_3$, 1%CH_3COOH, 0.005M HCl, 0.01M HCl, 0.1M HCl and milli-Q water were tested for the extraction process. The several parameters that influence on the extraction efficiency should be studied and optimized. Those factors were extraction solvent, weight of sample, volume of the extractant and extraction time. The extracted solutions were subjected to analysis of Fe by GFAAS. The optimum conditions in this study were obtained by the maximum value of iron content.
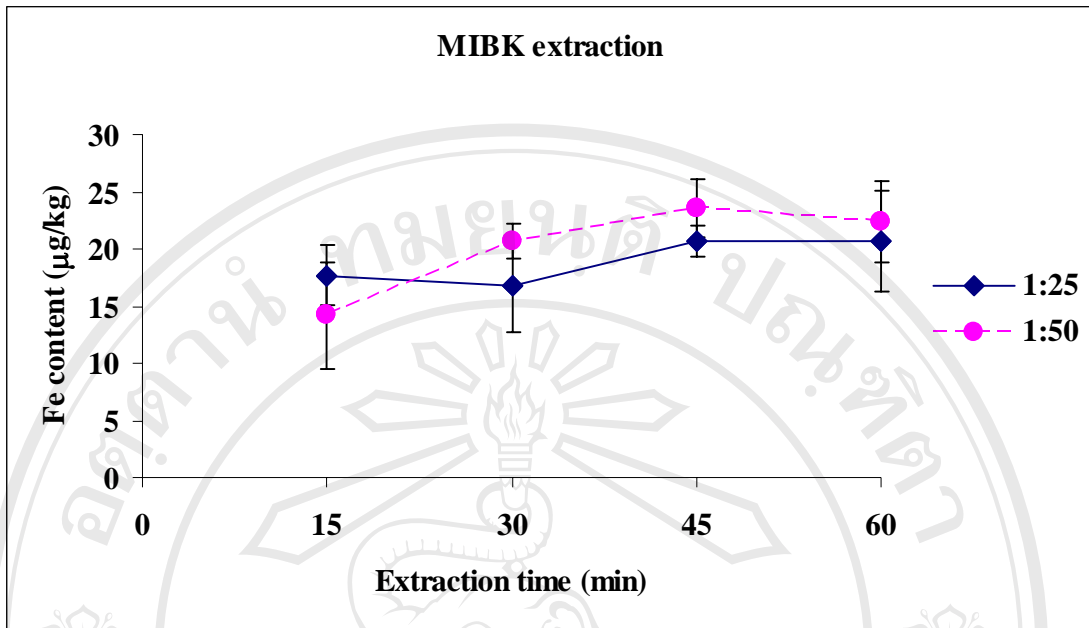
3.3.1 Optimization of MIBK extraction

The optimization of MIBK extraction were studied by varying the ratio of rice weight per MIBK volume at 1:25 and 1:50 g/ml and the extraction time from 15 to 60 min. The results are shown in **Table 3.6** and **Figure 3.3**. This indicated that the extracted Fe content were comparable for the ratio of rice weight per MIBK volume at 1:25 to 1:50 g/ml. The suitable ratio of rice weight per MIBK volume for the extraction was therefore chosen at 1:50 g/ml that was higher than 1:25 g/ml. The extracting volume and rice weight affect to the extractable Fe. Increasing extracting volume tends to slightly increase the extractable Fe. The optimum extraction time was 45 min in both of the ratios of rice weight per MIBK volume. Example of the calculation is shown in Appendix C.

Table 3.6 The effects of extraction time and the ratio of rice weight per MIBK volume on Fe extracted content

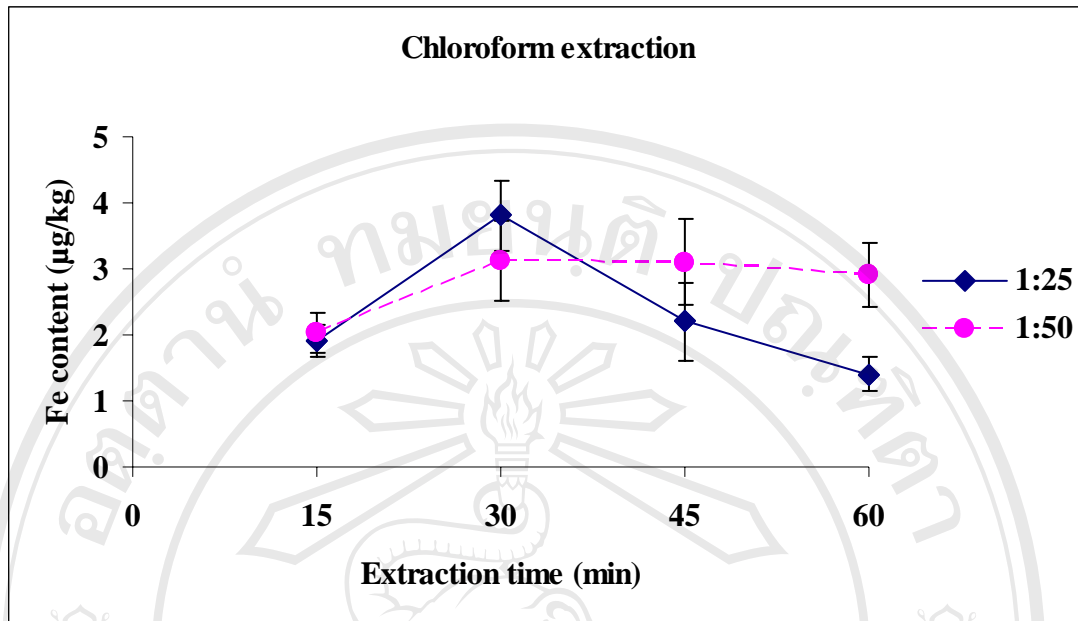| Extraction Time (min)	Rice weight per MIBK volume at 1:25 g/ml		Rice weight per MIBK volume at 1:50 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
15	17.7±2.6	14.9	14.2±4.7	16.0
30	16.8±4.0	23.9	20.7±2.5	10.8
45	20.7±1.4	6.6	23.6±1.5	7.3
60	20.7±4.4	21.2	22.4±3.6	33.2

Figure 3.3 The effects of extraction time and the ratio of rice weight per

MIBK volume on Fe extracted content.

3.3.2 Optimization of chloroform extraction

The optimization of chloroform extraction were studied by varying the ratio of rice weight per chloroform volume at 1:25 and 1:50 g/ml and the extraction time from 15 to 60 min. The results are shown in **Table 3.7** and **Figure 3.4**. This indicated that the extracted Fe content were comparable for the ratio of rice weight per chloroform volume at 1:25 to 1:50 g/ml. The suitable ratio of rice weight per chloroform volume for the extraction was therefore chosen at 1:25 g/ml because there were no significant different of the extractable Fe in both conditions and using less volume of solvent. Increasing extracting volume tended to increase the extractable Fe. The optimum extraction time was 30 min in both condition. Prolonging extraction time tended to decrease the extractable Fe because the volatilization of solvent had occurred easily. Therefore, chloroform was non-suitable for the extraction Fe in this case.

Table 3.7 The effects of extraction time and the ratio of rice weight per chloroform volume on Fe extracted content

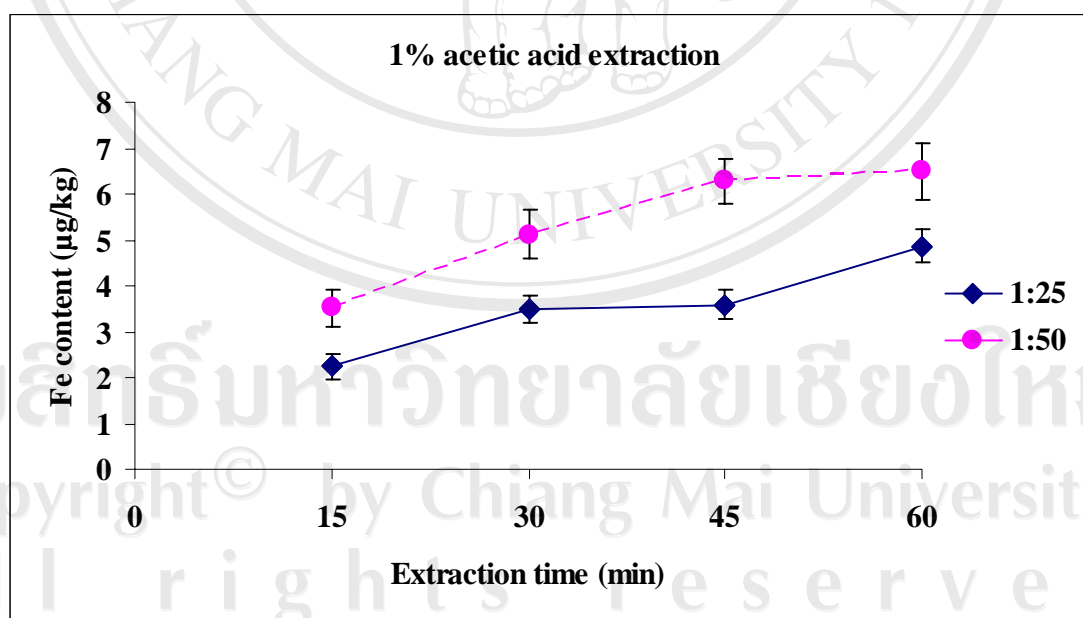| Extraction Time (min)	Rice weight per $CHCl_3$ volume at 1:25 g/ml		Rice weight per $CHCl_3$ volume at 1:50 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
15	1.9±0.2	12.5	2.9±0.5	14.6
30	3.8±0.5	14.1	3.1±0.7	19.5
45	2.2±0.6	27.0	3.1±0.6	21.3
60	1.4±0.3	17.7	2.0±0.3	16.7

Figure 3.4 The effects of extraction time and the ratio of rice weight per

chloroform volume on Fe extracted content.

3.3.3 Optimization of 1% acetic acid extraction

The optimization of 1% acetic acid extraction were studied by varying

the ratio of rice weight per 1% acetic acid volume at 1:25 and 1:50 g/ml and the

extraction time from 15 to 60 min. In **Table 3.8** and **Figure 3.5**, the result showed that

the extractable Fe content for the ratio of rice weight per 1% acetic acid volume at

1:25 were compared with 1:50 g/ml. The extractable Fe content from the ratio of 1:50

g/ml was higher than 1:25 g/ml, so that 1:50 g/ml was chosen. Increasing extracting

volume tends to increase the extractable Fe. The optimum extraction time in both was

60 min condition. The increasing of extractable Fe was obtained by increasing the

extraction time. In this case, the extraction time had more effect to the extractable Fe

than the other conditions.

Table 3.8 The effects of extraction time and the ratio of rice weight per 1% acetic

acid volume on Fe extracted content

| Extraction Time (min)	Rice weight per 1% acetic acid volume at 1:25 g/ml		Rice weight per 1% acetic acid volume at 1:50 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
15	2.2±0.3	13.1	3.5±0.4	11.4
30	3.5±0.3	8.5	5.1±0.5	10.3
45	3.6±0.3	9.0	6.3±0.5	7.6
60	4.8±0.3	7.2	6.5±0.6	9.4

Figure 3.5 The effects of extraction time and the ratio of rice weight per

1% acetic acid volume on Fe extracted content.

3.3.4 Optimization of 0.005M HCl extraction

The optimization of 0.005M HCl extraction were studied by varying the ratio of rice weight per 0.005M HCl volume 1:50 and 1:100 g/ml l and the extraction time from 2 to 24 hours. **Table 3.9** and **Figure 3.6** are indicated that the extractable Fe contents for the ratio of 1:100 g/ml are compared with 1:50 g/ml. The extractable Fe in the condition of 1:100 g/ml was higher than the condition of 1:50 g/ml. The optimum extraction time was 12 hours and 20 hours for 1:100 g/ml and 1:50 g/ml, respectively. Thus, the suitable optimum extraction time was 12 hours for the ratio of 1:100 g/ml because it was higher than 1:50 g/ml and used less extraction time. Longer extraction time slightly increases the extractable Fe.

Table 3.9 The effects of extraction time and the ratio of rice weight per 0.005M HCl volume on Fe extracted content

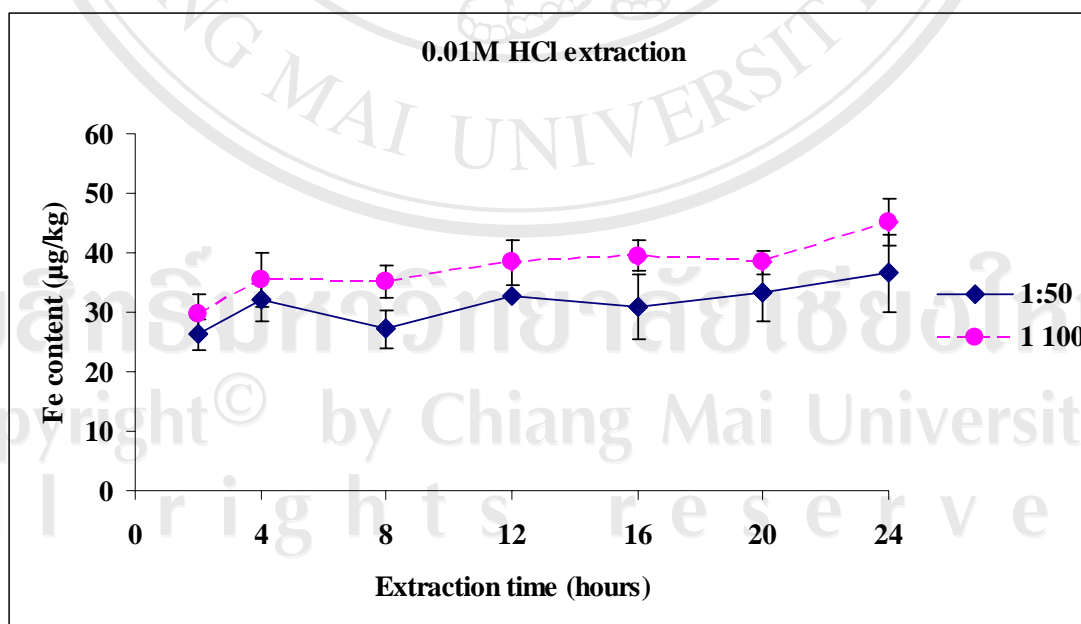| Extraction Time (min)	Rice weight per 0.005M HCl volume at 1:50 g/ml		Rice weight per 0.005M HCl volume at 1:100 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
2	32.6±3.9	11.9	30.5±4.3	14.2
4	39.9±4.7	11.7	38.1±2.2	5.7
8	33.8±4.4	13.1	45.8±2.4	5.2
12	46.1±2.9	6.3	63.6±4.4	6.9
16	47.1±3.1	6.4	57.4±4.2	7.2
20	61.7±2.1	3.4	66.5±2.4	3.6
24	59.3±4.9	8.2	67.6±6.7	9.9
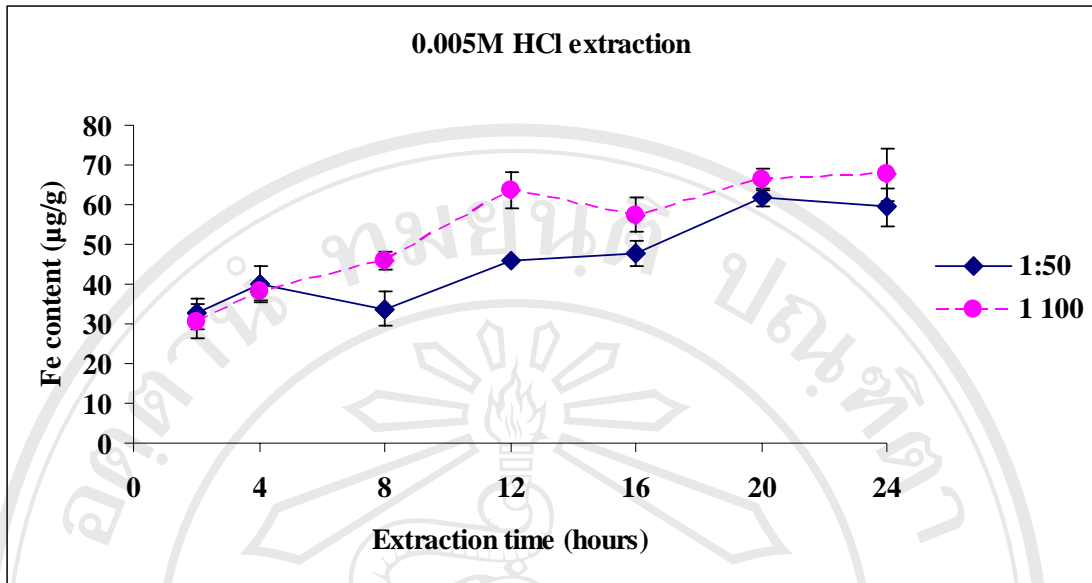
Figure 3.6 The effects of extraction time and the ratio of rice weight per

0.005M HCl volume on Fe extracted content.

3.3.5 Optimization of 0.01M HCl extraction

The optimization of 0.01M HCl extraction were studied by varying the ratio of

rice weight per 0.01M HCl volume of 1:50 and 1:100 g/ml l and the extraction time

from 2 to 24 hours. The results are shown in **Table 3.10** and **Figure 3.7**. This

provided that the extractable Fe contents were comparable for the ratio of rice weight

per 0.01M HCl volume at 1:50 to 1:100 g/ml. No signal increase was observed for the

extraction time longer than 12 hours. The optimum extraction time of the ratio of rice

weight per 0.01M HCl volume at 1:100 g/ml was higher than the other one.

Table 3.10 The effects of extraction time and the ratio of rice weight per 0.01M HCl

volume on Fe extracted content

Extraction Time (min)	Rice weight per 0.01M HCl volume at 1:50 g/ml		Rice weight per 0.01M HCl volume at 1:100 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
2	26.2±2.0	7.8	29.7±3.4	11.6
4	32.0±3.6	11.2	35.4±4.5	12.8
8	27.2±3.2	11.8	35.2±2.7	7.7
12	32.5±0.2	0.5	38.4±3.7	9.5
16	31.2±5.3	17.0	39.5±2.7	6.8
20	33.6±4.6	13.5	38.4±2.0	5.2
24	34.4±6.6	19.2	45.1±4.0	8.9

Figure 3.7 The effects of extraction time and the ratio of rice weight per
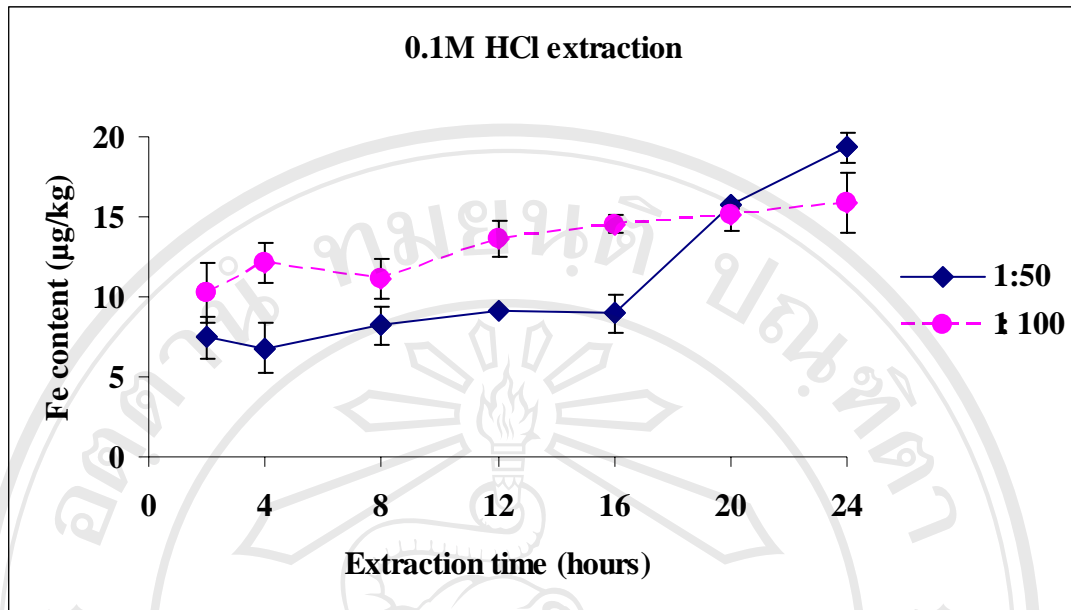
0.01M HCl volume on Fe extracted content.

3.3.6 Optimization of 0.1M HCl extraction

The optimization of 0.1M HCl extraction were studied by varying the ratio of rice weight per 0.1M HCl volume at 1:50 and 1:100 g/ml l and the extraction time from 2 to 24 hours. In **Table 3.11** and **Figure 3.8** are provide results for the ratio of rice weight per 0.1M HCl volume at 1:50 g/ml. The optimum extraction time was 24 hours for the ratio of 1:50 g/ml. The optimum extraction time was 12 hours for the ratio of 1:100 g/ml. Longer extraction time had no effect in the ratio of 1:100 g/ml. In contrast, sharply increase of the extractable Fe for the ratio of 1:50 g/ml was observed.

Table 3.11 The effects of extraction time and the ratio of rice weight per 0.1M HCl volume on Fe extracted content

Extraction Time (min)	Rice weight per 0.1M HCl volume at 1:50 g/ml		Rice weight per 0.1M HCl volume at 1:100 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
2	7.4±1.3	17.9	10.3±1.9	17.9
4	6.8±1.6	23.1	12.1±1.2	23.1
8	8.2±1.2	14.9	11.1±1.2	14.9
12	9.2±2.8	30.4	13.6±1.1	30.4
16	8.9±1.2	13.2	14.5±0.6	13.2
20	15.7±0.2	1.5	15.1±0.9	1.5
24	19.3±1.0	5.1	16.0±1.9	5.1

Figure 3.8 The effects of extraction time and the ratio of rice weight per

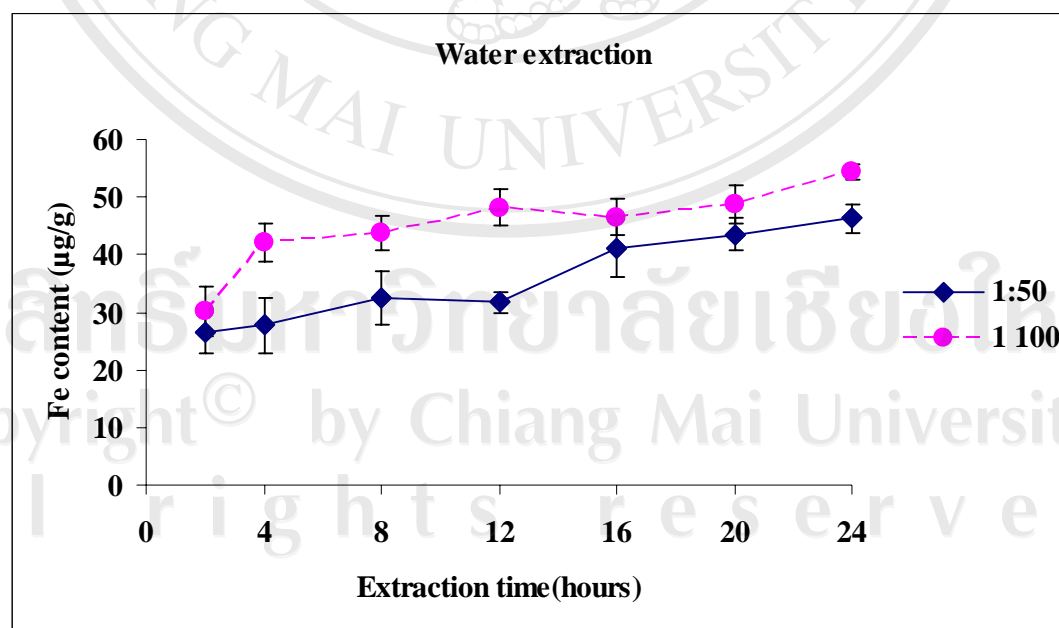0.1M HCl volume on Fe extracted content.

3.3.7 Optimization of water extraction

The optimization of water extraction were studied by varying the ratio of rice weight per water volume at 1:50 and 1:100 g/ml 1 and the extraction time from 2 to 24 hours. The results are shown in **Table 3.12** and **Figure 3.9**. The results illustrated that the ratio of rice weight per water volume 1:100 g/ml was obtained the extractable Fe higher than the ratio of 1:50 g/ml. Both conditions showed the best extraction time for 24 hours. The increasing in the extractable Fe was improved by the prolongation of the extraction time.

Most of the methods used to determine the iron content of several samples include an organic matter destruction step that modifies the oxidation state of the element. To study the oxidation state, water extraction must be applied to extract the soluble fraction of the element. Given that only the water soluble fraction of iron is available and that the most easily absorbable sub-fraction of it is Fe^{2+} form, it would be useful to differentiate in rice samples between soluble and insoluble iron, and in the soluble fraction between Fe^{2+} and Fe^{3+} in order to evaluate rice samples.

Table 3.12 The effects of extraction time and the ratio of rice weight per water volume on Fe extracted content

Extraction Time (min)	Rice weight per water volume at 1:50 g/ml		Rice weight per water volume at 1:100 g/ml	
	Mean±SD (N=3)	%RSD	Mean±SD (N=3)	%RSD
2	26.4±3.6	13.5	30.2±4.4	14.6
4	27.7±4.8	17.3	42.2±3.3	7.8
8	32.4±4.7	14.4	43.8±3.1	7.0
12	31.6±1.8	5.7	48.2±3.2	6.6
16	41.1±4.8	11.8	46.5±3.1	6.7
20	43.5±2.8	6.4	48.7±3.2	6.6
24	46.3±2.6	5.6	54.4±1.3	2.4

Figure 3.9 The effects of extraction time and the ratio of rice weight per water volume on Fe extracted content.

3.3.8 Results comparison

Summary of the suitable conditions of extraction method for the determination of extractable Fe content are shown in **Table 3.13**

Table 3.13 Suitable conditions of extraction methods

| Solvent | Optimum value | |
	The ratio of rice weight per solvent volume	Extraction time
MIBK	1:50 g/ml	45 min
CHCl$_3$	1:25 g/ml	30 min
1% CH$_3$COOH	1:50 g/ml	60 min
0.005M HCl	1:100 g/ml	12 hours
0.01M HCl	1:100 g/ml	12 hours
0.1M HCl	1:100 g/ml	12 hours
Milli-Q water	1:100 g/ml	24 hours

From **Table 3.13**, the optimum conditions were selected to determine the extractable Fe in Rice-3 samples. Results are shown in **Table 3.14** and **Figure 3.10**. The suitable solvent for extraction of Fe can be ordered as following;

Milli-Q water > 0.005M HCl > 0.01M HCl > 0.1M HCl > 1% CH$_3$COOH > MIBK > CHCl$_3$

Table 3.14 The content of Fe extracted by seven kinds of solvents

Solvent	Fe content* (μg/kg)	%RSD
MIBK	5.4±1.1	19.5
CHCl$_3$	1.6±0.5	31.8
1% CH$_3$COOH	7.1±0.6	9.1
0.005M HCl	101.2±10.8	10.7
0.01M HCl	42.1±5.0	12.0
0.1M HCl	32.9±3.8	11.6
Milli-Q water	133.7±19.8	14.8

* Mean±SD (N=3)
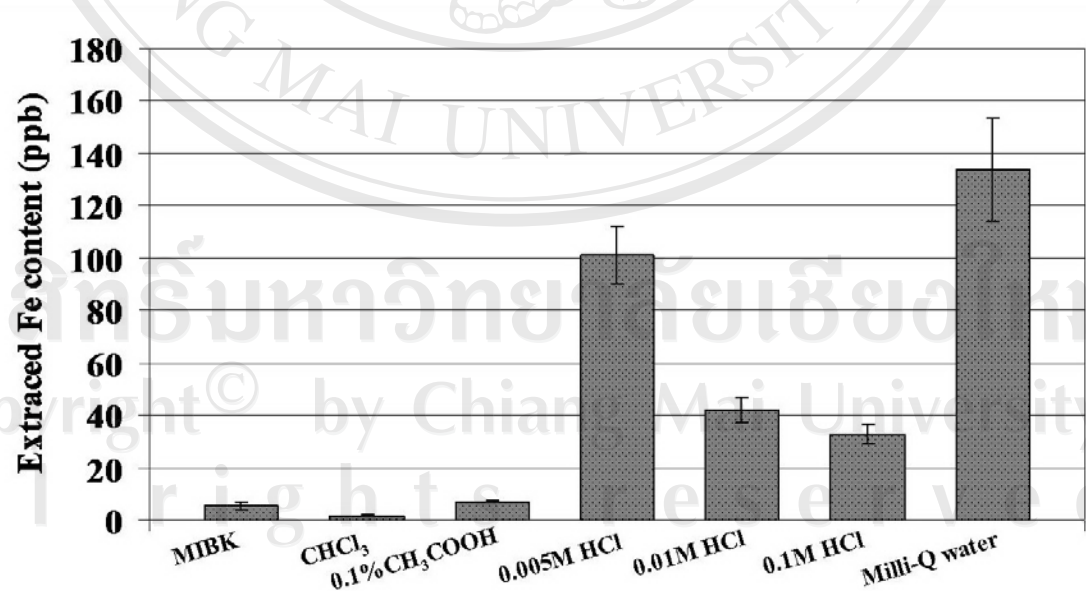
Figure 3.10 The comparison of the extractable Fe by seven kinds of solvents.

3.4 Optimization of speciation method

A method for speciation of ferrous and ferric ions was developed using solvent extraction and determined by GFAAS. The complexing reagent for Fe^{2+} in this work was 1-(2-pyridylazo)-2-naphthol (PAN). The Fe^{2+}–PAN complex was extracted into chloroform phase while Fe^{3+} remained in water phase. The organic phase containing Fe^{2+}-PAN complex was evaporated and the residue was redissolved with 4.0 ml of concentrated HNO_3 and then diluted with 2.0 ml of water. The resulting solution was subjected to analysis for Fe content by GFAAS.

The two significant advantages for the complexation are more sensitive for direct spectrophotometric detection and preservative of the original oxidation states which can be done by choosing a suitable complexing agents [7]. Although PAN has been used for the determination of iron using spectrophotometry, the other ions can interfere [42]. Using GFAAS after solvent extraction will readily eliminate these interferent effects sourced from other ions [13].

In order to perform the speciation of Fe^{2+} and Fe^{3+} from extracted solutions efficiently, several parameters that influence the extraction efficiency should be studied and optimized. Such factors included pH, extraction time, amount of PAN and volume of chloroform.

The studied conditions were optimized by mixed standard solution of 60 ppb Fe^{2+} and Fe^{3+}. The optimized separation conditions in this study were defined as the conditions which obtained the maximum recovery of Fe^{2+} in chloroform phase and the maximum recovery of Fe^{3+} in aqueous phase.

$$\text{\% recovery of } Fe^{2+} = \frac{\text{Abs. of spike } Fe^{2+} \text{ and } Fe^{3+} \text{ 60 ppb in CHCl}_3 \text{ phase}}{\text{Abs. of standard solution of 60 ppb } Fe^{2+}} \times 100$$

$$\text{\% recovery of } Fe^{3+} = \frac{\text{Abs. of spike } Fe^{2+} \text{ and } Fe^{3+} \text{ 60 ppb in aqueous phase}}{\text{Abs. of standard solution of 60 ppb } Fe^{3+}} \times 100$$

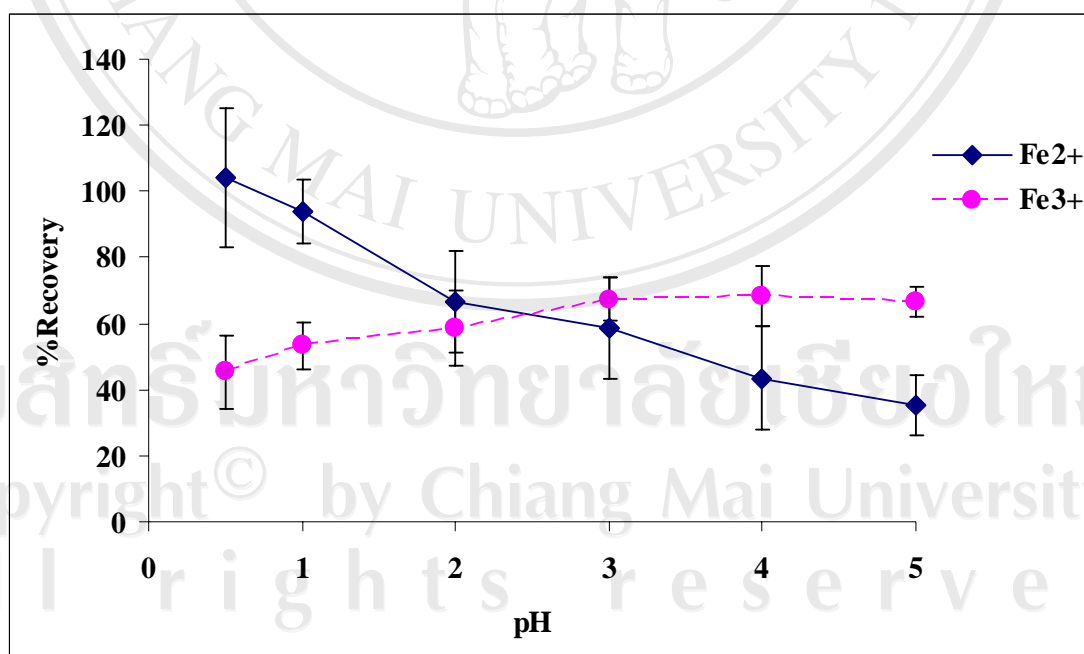### 3.4.1　The effect of pH on the recoveries of Fe^{2+} and Fe^{3+}

It is well known that the pH of the sample solution is one of the important factors affecting the formation of complexes. In this work, the optimization of pH was carried out in the range of 0.5-5.0 using 0.1 M HCl. The recoveries obtained for Fe^{2+} and Fe^{3+} at different pH values are given in **Table 3.15** and **Figure 3.11**. The maximum recoveries of Fe^{2+} were observed at pH 0.5-1.0 and then the recoveries values decreased with the increasing of pH. However, the recoveries of Fe^{3+} increased at pH 2. The optimum pH 1.0 was chosen due to the maximum recovery of Fe^{2+}. The calculation of recoveries is shown in Appendix C. The hard/soft-acid/base (HSAB) principle is widely used in chemistry for explaining stability of compounds, reaction mechanisms and pathways [43]. Hard applies to species which are small, have high charge states, and are weakly polarizable that is Fe^{3+} specie in the solution. But Soft applies to species which are big, have low charge states and are strongly polarizable that is Fe^{2+} specie in the solution. The gist of this theory is that soft acids react faster and form stronger bonds with soft bases, whereas hard acids react faster with hard bases. From the result was shown that PAN (soft base) form bonds with Fe^{2+} (soft acid) that can be explained by the HSAB principle.

Table 3.15 The effect of pH on the percent recovery of Fe^{2+} and Fe^{3+}

pH	Fe^{2+}		Fe^{3+}	
	% Recovery*	%RSD	% Recovery*	%RSD
0.5	104.2±21.2	20.3	45.5±11.1	24.4
1	94.0±9.7	10.3	53.4±7.1	13.4
2	66.7±15.4	23.1	58.6±11.4	19.4
3	58.6±15.4	26.2	67.3±6.7	10.0
4	43.4±15.7	36.2	68.4±9.2	13.5
5	35.3±9.2	26.2	66.4±4.5	6.8

* Mean±SD (N=3)

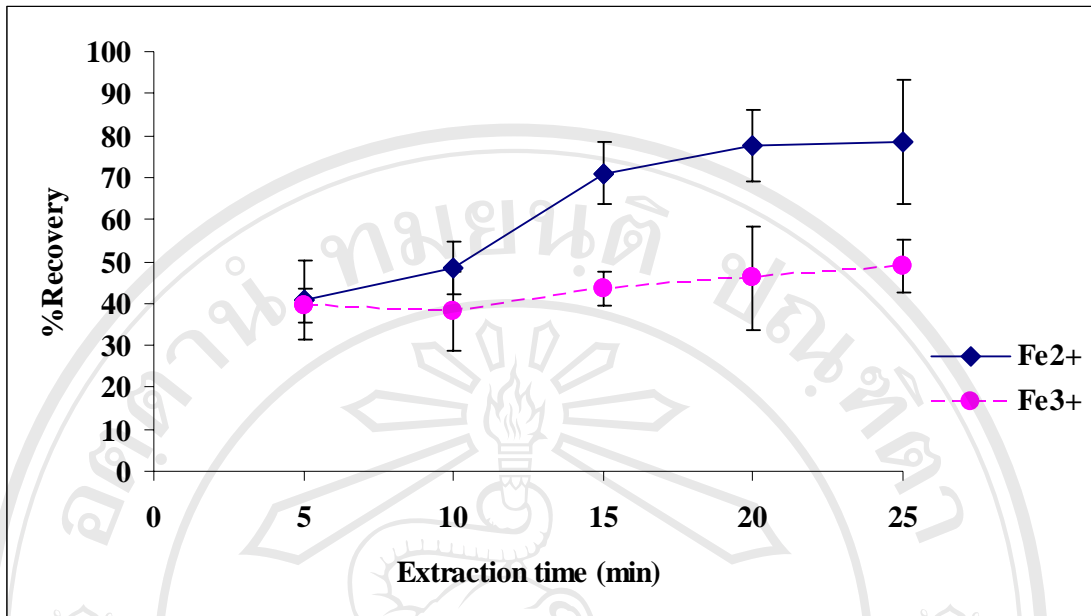**Figure 3.11** The effect of pH on the percent recovery of Fe^{2+} and Fe^{3+}.

3.4.2 The effect of extraction time on the recoveries of Fe^{2+} and Fe^{3+}

A sufficient extraction time is necessary to attain equilibrium of analytes between the aqueous phase and the organic phase. To extract the maximum amount of analytes so, the effect of extraction time in the yield of the solvent extraction was optimized. In this work, the optimization of the extraction time was examined in the range of 5-30 min. From **Table 3.16** and **Figure 3.12**, the recoveries of Fe^{2+} and Fe^{3+} increased when the extraction time was varied from 5 to 15 min and remained constant from 15 to 25 min. On the other hand, the extraction time at 30 min, the phase separation was difficult to obtained. So, the extraction time at 15 min was chosen considering the less extraction time and ease of phase separation.

Table 3.16 The effect of extraction time on the percent recovery of Fe^{2+} and Fe^{3+}

| Extraction time	Fe^{2+}		Fe^{3+}	
(min)	% Recovery*	%RSD	% Recovery*	%RSD
5	40.8±9.5	23.4	39.5±4.1	10.4
10	48.5±6.3	13.1	38.2±9.4	24.7
15	71.0±7.4	10.5	43.4±4.0	9.1
20	77.6±8.5	11.0	46.0±12.2	26.5
25	78.4±14.8	18.9	48.9±6.4	13.1

* Mean±SD (N=3)

Figure 3.12 The effect of extraction time on the percent recovery of Fe^{2+} and Fe^{3+}.

3.4.3 The effect of the amount of PAN on the recoveries of Fe^{2+} and Fe^{3+}

1-(2-pyridylazo)-2-naphthol (PAN), a pyridylazo reagent, reacts with many metallic ions, producing colored complexes that are usually insoluble in water but soluble in organic reagents, like chloroform, benzene and carbon tetrachloride [44]. Their stability is greatly affected by the acidity. The alkali and alkaline earth metals do not form colored chelates with PAN. Therefore, PAN is used as colorimetric reagent for the quantitative and qualitative determination of variety of metal ions. The structure of PAN is shown in **Figure 3.13**.

The reaction may be written as follows [45]:
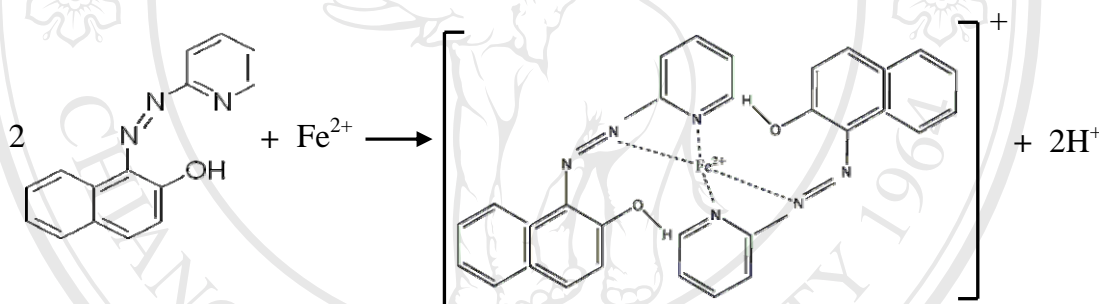
A sufficient ligand is needed for the complex formation of trace metal ions under optimum conditions. The influence of the amount of PAN is also evaluated and the results are shown in **Table 3.17** and **Figure 3.14**. Increasing the %PAN (w/v), the recoveries were slightly increased. Hence, 0.1%PAN (w/v) was selected because at higher %PAN (w/v), the excess amount of PAN will react with Fe^{3+} that cause the determination of Fe was over.

Table 3.17 The effect of PAN amount on the percent recovery of Fe^{2+} and Fe^{3+}

%PAN	Fe^{2+}		Fe^{3+}	
(w/v)	% Recovery*	%RSD	% Recovery*	%RSD
0.1	76.5±11.1	14.5	32.9±9.0	27.2
0.2	83.5±15.0	18.0	28.5±5.9	20.8
0.3	106.1±12.0	11.3	21.9±3.7	17.0
0.4	138.4±9.2	6.7	18.2±5.7	31.3

* Mean±SD (N=3)
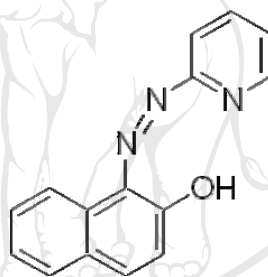
Figure 3.13 The structure of PAN [46].
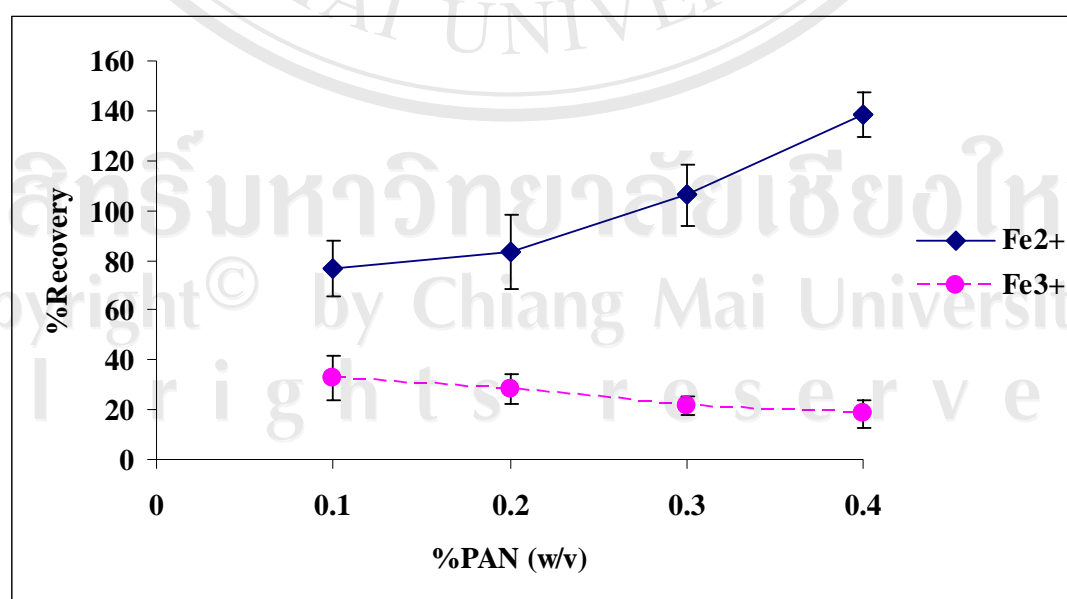
Figure 3.14 The effect of amount of PAN on the percent recovery of Fe^{2+} and Fe^{3+}.
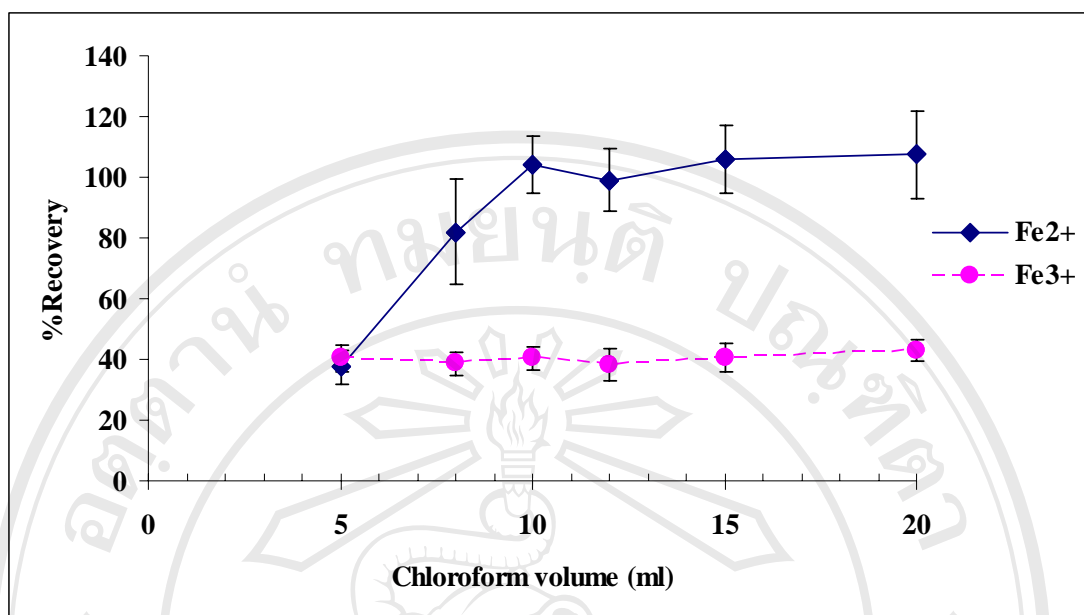
3.4.4 The effect of the chloroform volume on the recoveries of Fe^{2+} and Fe^{3+}

The influence of the chloroform volume was evaluated. The results are shown in **Table 3.18** and **Figure 3.15**. The recoveries were investigated by varying the volume of chloroform in the range of 5 to 20 ml. From the results, we can found that the best volume of chloroform used was 10 ml because the recovery was higher than the volume of chloroform 5 and 8 ml. The recoveries were constant when using higher volume of chloroform at 15 and 20 ml was used.

Table 3.18 The effect of chloroform volume on the percent recovery of Fe^{2+} and Fe^{3+}

Chloroform Volume	Fe^{2+}		Fe^{3+}	
(ml)	% Recovery*	%RSD	% Recovery*	%RSD
5	37.4±5.4	14.4	40.4±4.6	11.3
8	82.0±17.2	21.0	38.5±3.9	10.0
10	104.1±9.3	8.9	40.4±3.6	9.0
12	98.9±10.3	10.4	38.0±5.2	13.8
15	105.8±11.2	10.6	40.5±4.7	11.5
20	107.4±14.6	13.6	42.8±3.4	7.9

* Mean±SD (N=3)

Figure 3.15 The effect of chloroform volume on the percent recovery of Fe^{2+}

and Fe^{3+}.

Summary of the optimum conditions of speciation method for the determination of Fe^{2+} and Fe^{2+} are shown in **Table 3.19**

Table 3.19 Optimum conditions of speciation methods

Parameter	Optimum value
pH	1.0
Extraction time (min)	15
% PAN (w/v)	0.1
Chloroform volume (ml)	10

3.5 Determination of Ferrous and Ferric ions in Rice Samples

The optimized method was applied to the determination of Fe^{2+} and Fe^{3+} in the rice bran samples. The results obtained were given in **Table 3.20**. The content of Fe^{3+} was calculated by the subtraction of Fe^{2+} from the extracted Fe content which was determined by direct measurement of GFAAS. The content of Fe^{3+} was also calculated by the equation below:

$$\text{Extracted Fe} = Fe^{2+} + Fe^{3+}$$

Table 3.20 The contents of extracted Fe, Fe^{2+}, Fe^{3+} and total Fe in rice bran samples by GFAAS method

Sample	Extracted Fe [A] (µg/kg)	Fe^{2+} [B] (µg/kg)	Fe^{3+} [C] (µg/kg)	Total Fe [D] (µg/kg)
Rice bran-1	41.3±0.01	31.6±9.4	9.68	92.0±5.6
Rice bran-2	77.7±0.01	50.4±6.6	27.34	160.8±14.4
Rice bran-3	111.2±0.01	83.4±7.2	27.73	120.8±23.5
Rice bran-4	67.4±0.01	67.2±4.2	0.24	101.5±13.7
Rice bran-5	78.7±0.01	51.6±8.0	27.02	138.3±8.1

[A] Mean±SD, Three replicated injections
[B] Mean±SD, Three reproducibility
[C] Calculated from equation : Extracted Fe $=$ Fe^{2+} $+$ Fe^{3+}
[D] Mean±SD, Three reproducibility

Table 3.21 The contents of extracted Fe in rice bran samples by ICP-MS method

Sample	Fe (µg/kg)	Mg (µg/kg)	Cu (µg/kg)	Zn (µg/kg)	Cd (mg/kg)	Pb (mg/kg)
Rice bran-1	50.8±7.8	122.8±0.6	46.7±0	679.0±0	< 0 ppm	< 0 ppm
Rice bran-2	78.0±3.3	122.4±3.7	69.6±0	885.4±0	< 0 ppm	< 0 ppm
Rice bran-3	90.5±0	150.3±0.4	38.0±0	860.8±0	< 0 ppm	< 0 ppm
Rice bran-4	62.1±0.1	124.7±0.1	55.0±0	794.3±0	< 0 ppm	< 0 ppm
Rice bran-5	72.6±0.2	118.3±2.5	94.2±0	999.2±0	< 0 ppm	< 0 ppm

Mean±SD, Two replicated injections