

APPENDIX A

Calibration curve for determination of iron by GFAAS

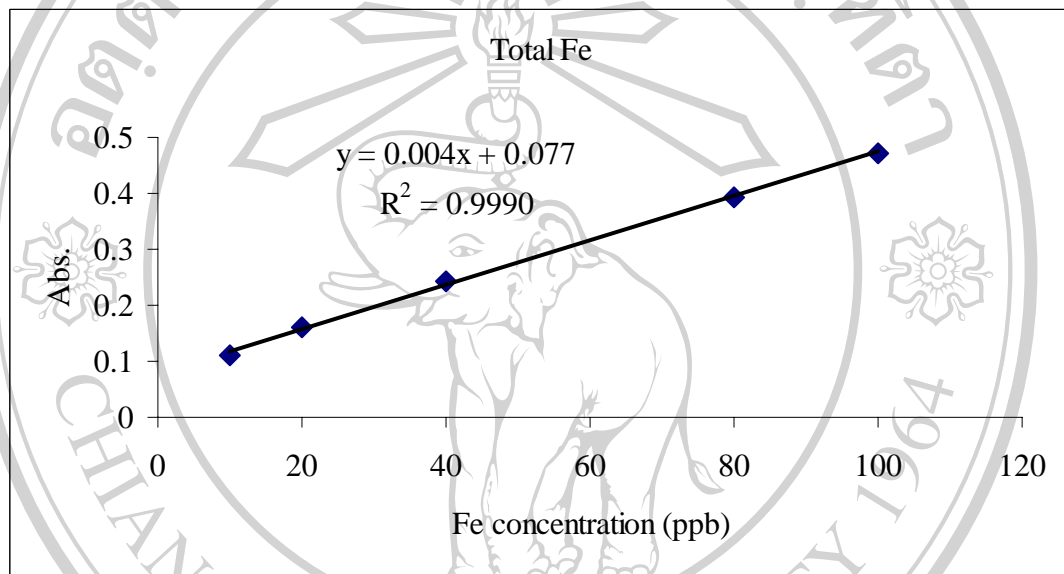


Figure A-1 Calibration curves of total Fe content in rice and rice bran samples obtained from GFAAS

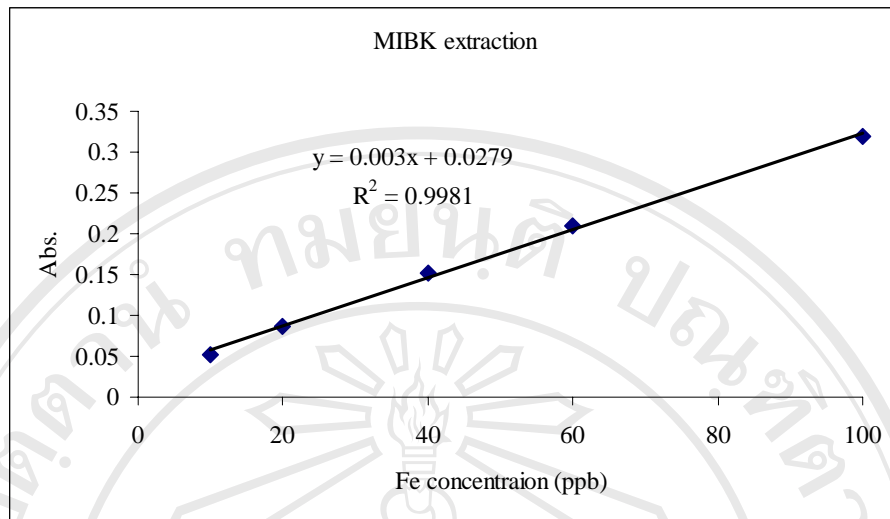


Figure A-2 Calibration curves of extracted Fe for MIBK extraction obtained from GFAAS

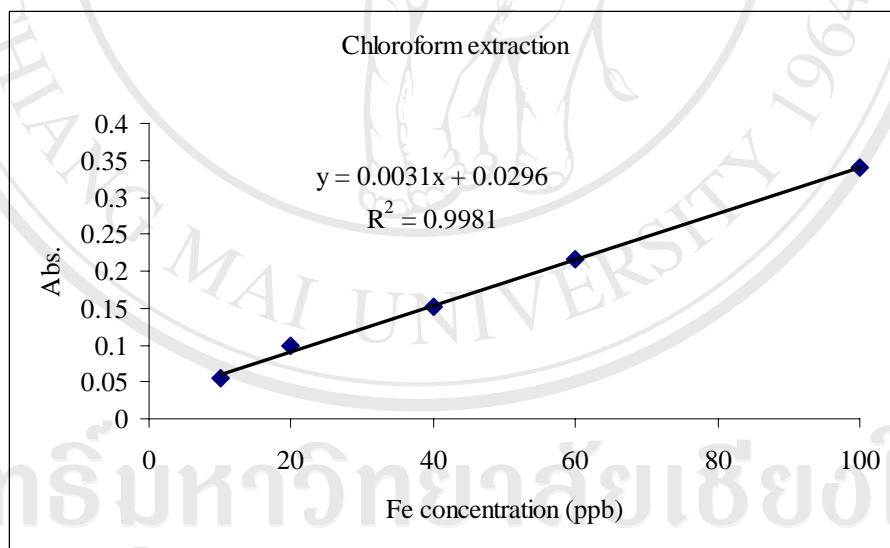


Figure A-3 Calibration curves of extracted Fe for chloroform extraction obtained from GFAAS

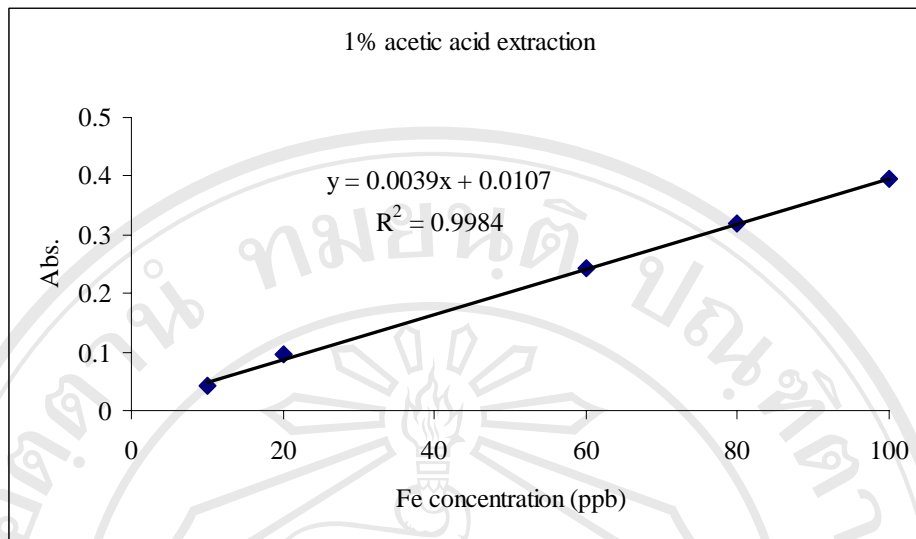


Figure A-4 Calibration curves of extracted Fe for 1% acetic acid extraction obtained from GFAAS

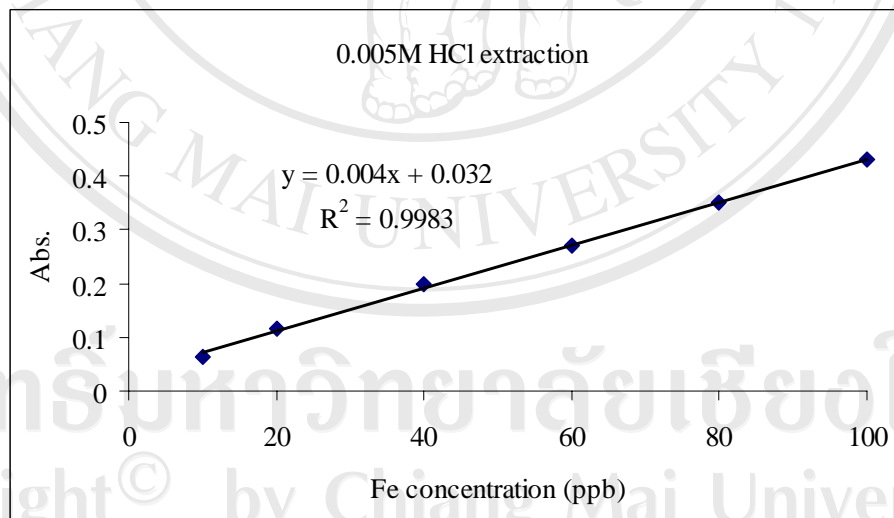


Figure A-5 Calibration curves of extracted Fe for 0.005M HCl extraction obtained from GFAAS

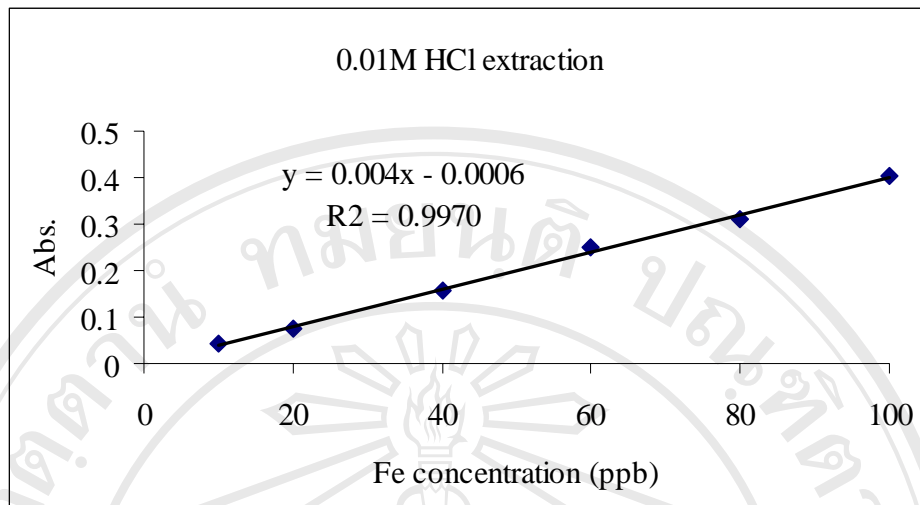


Figure A-6 Calibration curves of extracted Fe for 0.01M HCl extraction obtained from GFAAS

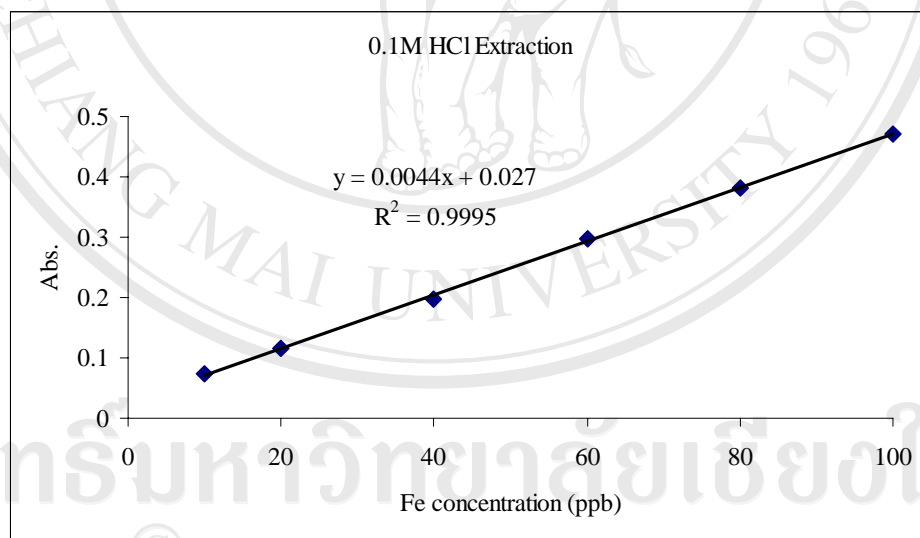


Figure A-7 Calibration curves of extracted Fe for 0.1M HCl extraction obtained from GFAAS

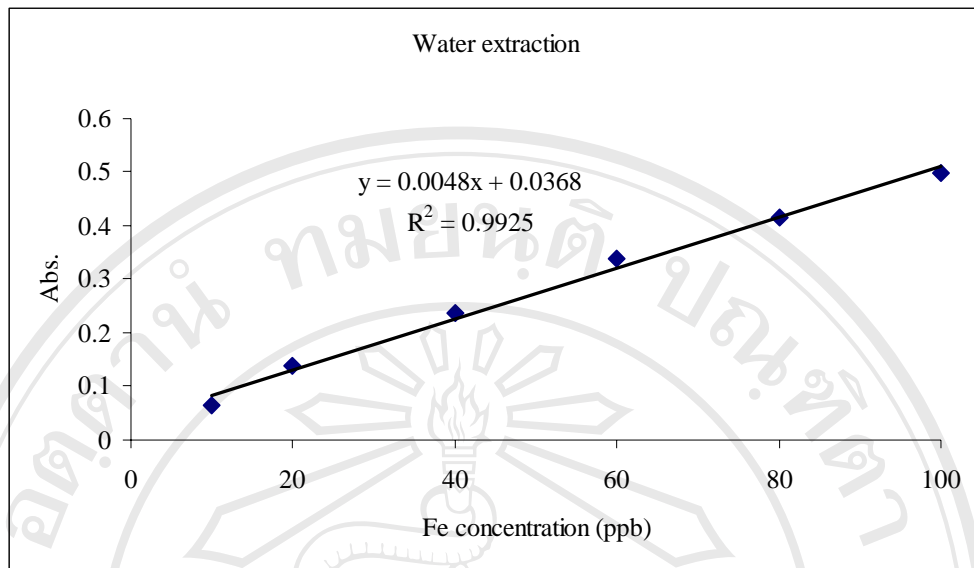


Figure A-8 Calibration curves of extracted Fe for water extraction obtained from GFAAS

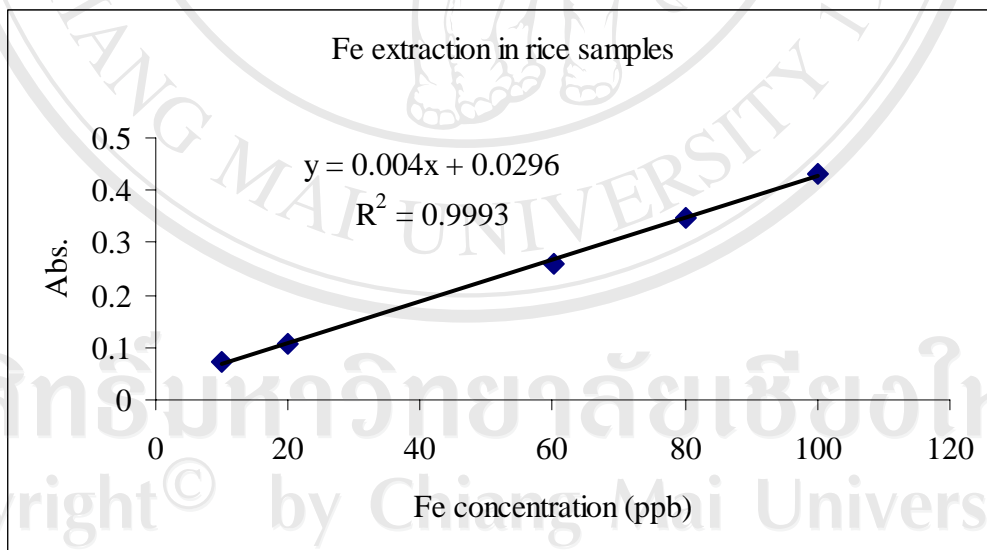
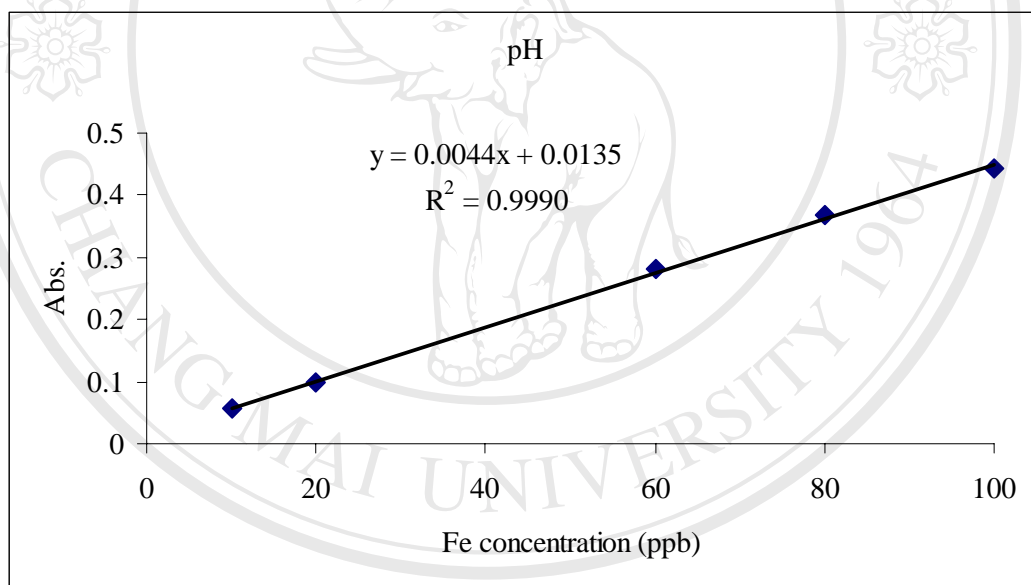
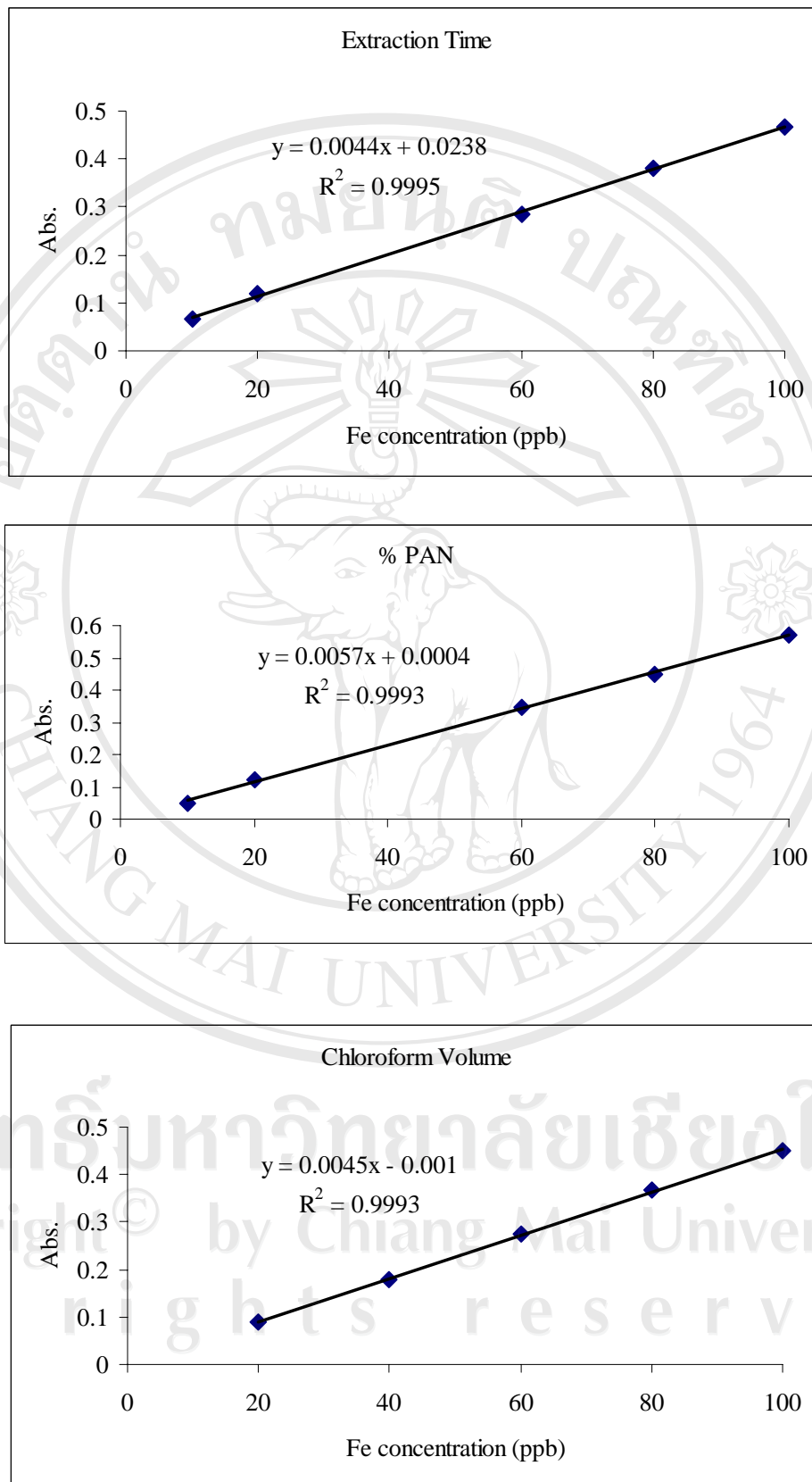


Figure A-9 Calibration curves of Fe for extraction method in rice samples

Table A-1 Calibration curve data of Fe for speciation method obtained from GFAAS

Parameter	Regression line equation	Correlation coefficient (R^2)
pH	$y = 0.0044x + 0.0135$	0.9990
Extraction time (min)	$y = 0.0044x + 0.0238$	0.9995
% PAN (w/v)	$y = 0.0057x + 0.0004$	0.9993
Chloroform volume (ml)	$y = 0.0045x - 0.001$	0.9993

**Figure A-10** Calibration curve data of Fe for speciation method

**Figure A-10 (Continued)**

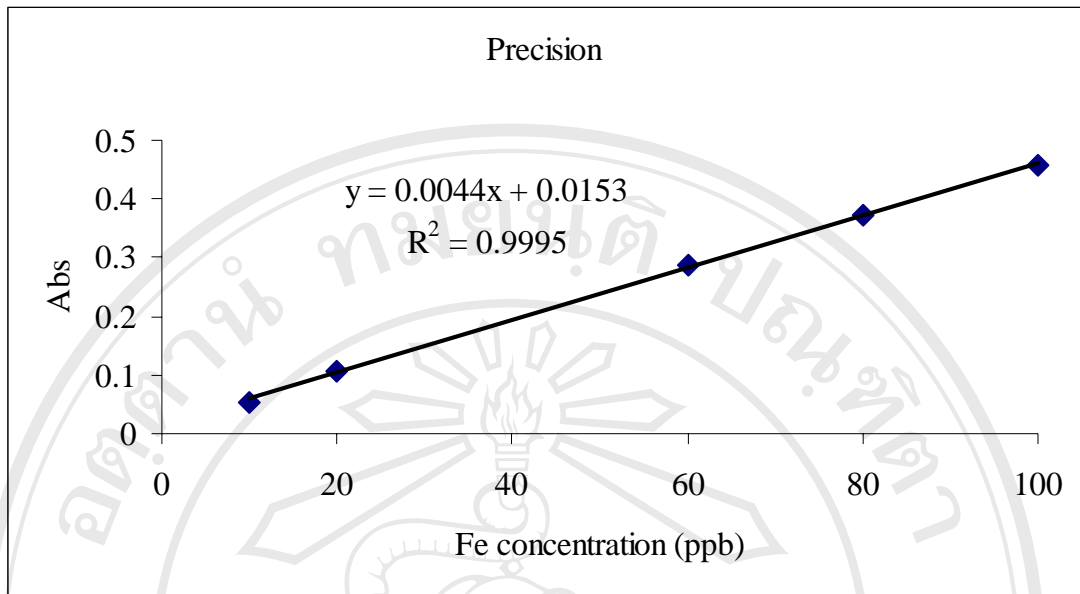


Figure A-11 Calibration curves of Fe for precision analysis

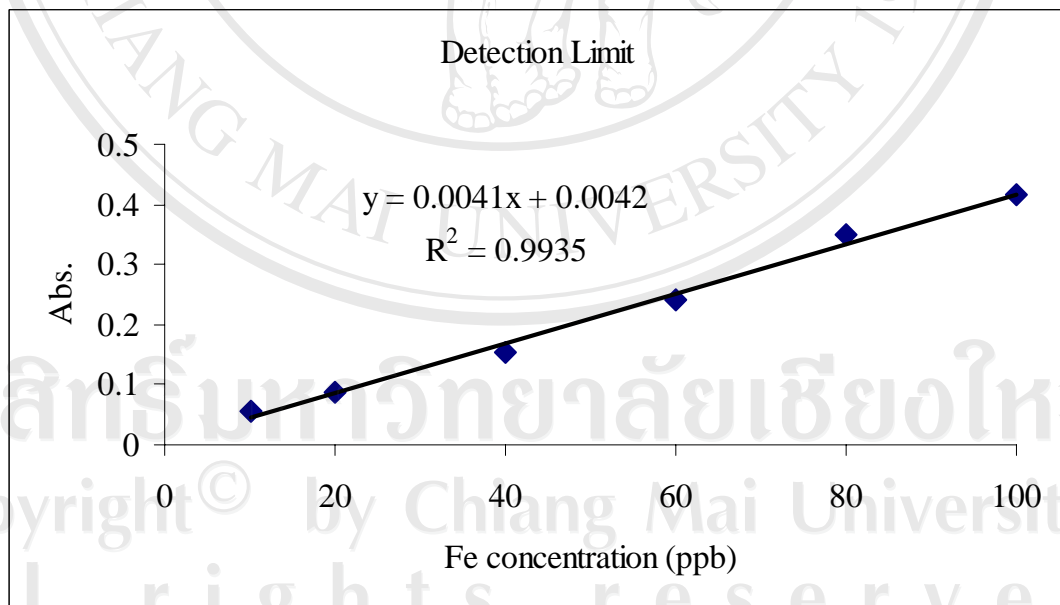


Figure A-12 Calibration curves of Fe for detection limit analysis

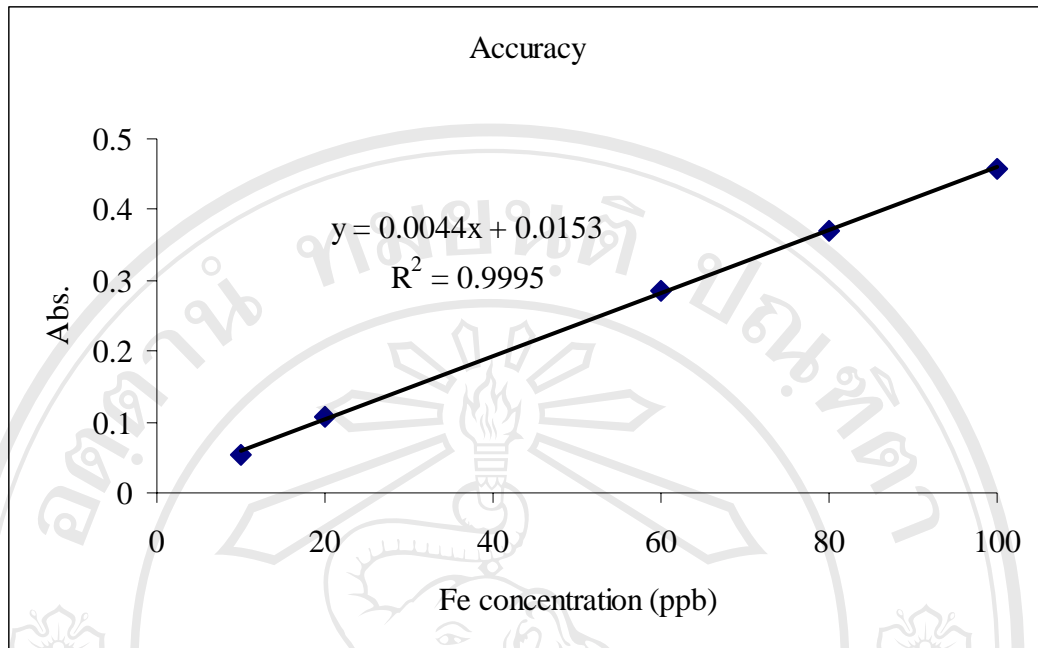


Figure A-13 Calibration curves of Fe for accuracy

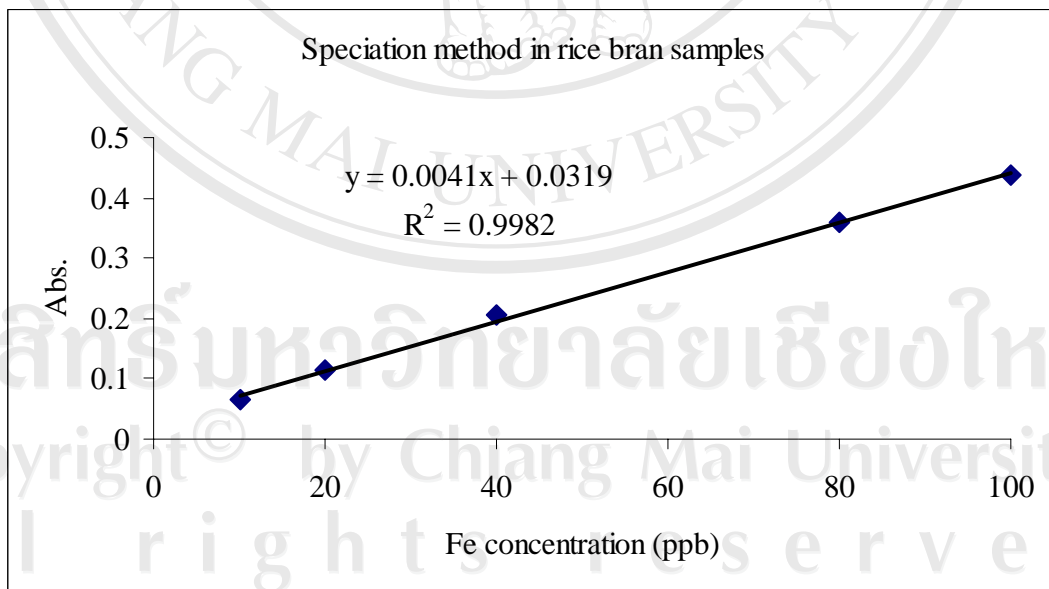


Figure A-14 Calibration curves of Fe for speciation method in rice bran samples

APPENDIX B

B-1 Standard deviation [47-48]

The most common measure of the error or a statistical measure of precision in an experimental quantity is standard deviation (SD) of a set of data. SD defines a series of n measurements of the same measure and, the quantity is characterizing the distribution of the results and given by the formula:

$$SD = \left[\sum (X_i - \bar{X})^2 / (n-1) \right]^{1/2}$$

Where

X_i is the result of the i measurement

\bar{X} is the arithmetic mean of the n results considered

n is the number of measurement

The definition is estimated the standard deviation for n values of a sample of a population and is always calculated using n-1. If the analysis was repeated several times to produce several sample sets of data, it would be expected that each set of measurements would have a different mean and a different estimate of the standard deviation.

B-2 Relative standard deviation [47-48]

The most useful test parameter is the precision of replicate injections of the analytical reference solution, prepared as directed under the individual reagent. The precision of replicate injections is expressed in term of relative standard deviation as follows:

$$\%R.S.D. = (SD / \bar{X}) \times 100$$

Where

SD is standard deviation

\bar{X} is the mean of data

B-3 The detection limit [49]

The detection limit is the minimum concentration of analyte that can be detected and can be calculated from following equation.

$$\text{Detection limit} = (3 \times SD) / \text{slope}$$

Where

3 is Z-value at 99% confidence level

SD is the standard deviation of blank

Slope is slope of calibration curve

APPENDIX C

C-1 The concentration of total Fe in rice sample by microwave method and GFAAS

Rice sample solution was extracted from 0.5 g of rice in 25 ml of milli-Q water. The extracted Fe in the sample solution was 10045 ppb which the concentration of Fe was determined by GFAAS.

1000 ml of sample solution contains Fe 10045 mg

25 ml of sample solution contains Fe $\frac{10045 \times 25}{1000}$ mg

0.5 g of rice sample contains Fe $\frac{10045 \times 25}{1000}$ mg

So, 1 g of rice sample contains Fe $\frac{10045 \times 25}{1000 \times 0.5} = 502.2$ mg

or 1 g of rice sample contains Fe 502.2 ppb

The concentration of extracted Fe in the other rice samples can be calculated similar to this way.

C-2 The concentration of extracted Fe in rice sample

Rice sample solution was extracted from 2 g of rice in 100 ml of MIBK.

The extracted Fe in the sample solution was 282.50 ppb which the concentration of Fe was determined by GFAAS.

1000 ml of sample solution contains Fe $282.50 \mu\text{g}$

100 ml of sample solution contains Fe $\frac{282.50 \times 100}{1000} \mu\text{g}$

2 g of rice sample contains Fe $\frac{282.50 \times 100}{1000} \mu\text{g}$

So, 1 g of rice sample contains Fe $\frac{282.50 \times 100}{1000 \times 2} = 14.1 \mu\text{g}$

or 1 g of rice sample contains Fe 14.1 ppb

The concentration of extracted Fe in the other rice samples can be calculated similar to this way.

C-3 The percent recoveries of Fe²⁺ and Fe³⁺ from speciation method

Table C-1 The effect of pH on the percent recovery of Fe²⁺

pH	D.F.	Abs.	Conc.	% recovery	Mean	SD	%RSD
0.5	2	0.2105	0.4210	88.2	104.2	21.2	20.3
	2	0.2685	0.5370	128.2			
	2	0.2222	0.4444	96.3			
1	2	0.2053	0.4106	84.6	94.0	9.7	10.3
	2	0.2334	0.4668	104.0			
	2	0.2182	0.4364	93.5			
2	2	0.1821	0.3642	68.6	66.7	15.4	23.1
	2	0.2002	0.4004	81.1			
	2	0.1558	0.3116	50.4			
3	2	0.1772	0.3544	65.2	58.6	15.4	26.2
	2	0.1422	0.2844	41.1			
	2	0.1836	0.3672	69.6			
4	2	0.1702	0.3404	60.4	43.4	15.7	36.2
	2	0.1412	0.2824	40.4			
	2	0.1253	0.2506	29.4			
5	2	0.1481	0.2962	45.1	35.3	9.2	26.2
	2	0.1216	0.2432	26.8			
	2	0.1317	0.2634	33.8			

Calibration curve: $y = 0.0044x + 0.0135$

$$R^2 = 0.9990$$

The absorbance of blank was 0.1654

The absorbance of standard solution of Fe²⁺ 60 ppb was 0.2898

pH 0.5, Abs. = 0.4210 – blank = 0.4210 – 0.1654 = 0.2556

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

$$\% \text{ recovery for Fe}^{2+} = (0.2556/0.2898) \times 100 = 88.2$$

The % recovery for Fe²⁺ and Fe³⁺ in the other samples can be calculated similar to this way.

C-4 The detection limit

The detection limit of the method for determination of iron was investigated by analyzing blank (0.2% HNO₃) ten times.

Table C-2 Absorbance obtained for blank solution.

0.2%HNO ₃	Abs.
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

$$\text{Detection limit} = (3 \times \text{SD}) / \text{slope}$$

$$\text{Detection limit} = (3 \times 0.0004) / 0.0041 = 0.2882 \text{ ppb}$$

C-5 The accuracy

The method was examined by determining the recoveries of the added 60 ppb of Fe²⁺ and Fe³⁺ in sample solution. The results are shown in **Table C-3**.

Table C-3 The spike sample results

Sample	D.F.	Abs.	Conc.	Conc. x D.F.	Cal	%recovery	mean	SD	%RSD
Rice bran-1	10	0.4017	87.81	878.18	87.82	105	114	8.8	7.8
	10	0.4247	93.04	930.45	93.05	114			
	10	0.4484	98.43	984.31	98.43	123			
Rice bran-2	20	0.3210	69.47	1389.54	138.95	81.9	83.4	12.4	14.9
	20	0.3402	73.84	1476.81	147.68	96.4			
	20	0.3076	66.43	1328.63	132.86	71.7			
Rice bran-3	20	0.3212	69.52	1390.45	139.05	122	115	6.4	5.6
	20	0.3109	67.18	1343.63	134.36	114			
	20	0.3043	65.68	1313.63	131.36	109			
Rice bran-4	20	0.3243	70.22	1404.54	140.45	116	113	6.4	5.6
	20	0.3103	67.04	1340.90	134.09	105			
	20	0.3254	70.47	1409.54	140.95	117			
Rice bran-5	20	0.3465	75.27	1505.45	150.55	92.8	76.3	15.1	19.8
	20	0.3072	66.34	1326.81	132.68	63.0			
	20	0.3206	69.38	1387.72	138.77	73.1			

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Table C-4 The sample results

Sample	D.F.	Abs.	Conc.	Conc. x D.F.	Cal
Rice bran-1	10	0.1243	24.7727	247.72	24.77
Rice bran-2	10	0.4105	89.8182	898.18	89.82
Rice bran-3	10	0.3056	65.9773	659.77	65.98
Rice bran-4	10	0.3267	70.7727	707.72	70.77
Rice bran-5	10	0.4328	94.8864	948.86	94.89

Rice bran-1,

$$\% \text{ recovery} = \frac{\text{spike sample result} - \text{sample result}}{\text{spike amount added}} \times 100$$

$$\% \text{ recovery} = \frac{87.82 - 24.77}{60} \times 100$$

$$\% \text{ recovery} = 105$$

The concentration of % recovery in the other rice samples can be calculated similar to this way.

C-6 The concentration of Fe^{2+} in rice sample by speciation method

Rice sample solution was extracted from 2 g of rice in 200 ml of water then, 25 ml of sample solution was pipetted into vessel. 0.5 ml of 0.1% PAN and 10 ml of CHCl_3 were added into the sample solution, respectively. The layer of CHCl_3 was separated. Finally 4 ml of HNO_3 and 2 ml of water were added into the separated solution for determination of Fe by GFAAS.

The absorbance of Fe^{2+} from calibration curve was 404.4 ppb

1000 ml of sample solution contains Fe^{2+} 404.4 μg

25 ml of sample solution contains Fe^{2+} $\frac{404.4 \times 25}{1000 \times 25}$ μg

1 ml of sample solution contains Fe^{2+} $\frac{404.4 \times 25}{1000 \times 25} = 0.4044$ μg

200 ml of sample solution contains Fe^{2+} 0.4044×200 μg

2 g of rice sample contains Fe^{2+} 0.4044×200 μg

So, 1 g of rice sample contains Fe^{2+} $\frac{0.4044 \times 200}{2} = 40.4$ μg

or 1 g of rice sample contains Fe^{2+} 40.4 ppb

The concentration of Fe^{2+} in the other rice samples can be calculated similar to this way.

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- National presentation**
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- Preliminary Study of Quantitative Determination of Iron, Zinc, Copper and Manganese in Rice Samples by Atomic Absorption Spectrophotometry, 32nd Congress on Science and Technology of Thailand at Queen Sirikit National Convention Center, October 10-12, 2006
- Preliminary Study on Iron in Rice Samples by Graphite Furnace Atomic Absorption Spectrometry (GFAAS), 5th PERCH-CIC Annual Scientific Congress, Jomtien Palm Beach Resort, Pattaya, Chonburi, Thailand, May 6-9, 2007

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THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Rice is the staple food for over half of the world's population. In addition, it is the major source of some micronutrients for Thai people. Iron is an essential nutritional element for all life forms, e.g. it is a cofactor in many enzymes and essential for oxygen transport and electron transfer. The fractional absorption of iron was correlated with its oxidation state. Also, Fe^{2+} is required for proper transport and storage of oxygen in higher animal by means of hemoglobin and myoglobin, while oxidized forms methemoglobin and metmyoglobin, which contain Fe^{3+} , will not bind oxygen. Iron absorption refers to the amount of dietary iron that the body obtains from the intake food. Insufficient iron level is causally related to iron-deficiency anemia (IDA). Anemia is a condition in which the blood cannot carry enough oxygen because there are less red blood cells than normal. Metal speciation is important in a variety of environmental, biological, geological and medical applications. The chemical and physical properties of metal species highly depend on its oxidation state, hence an accurate determination of each species is important to evaluate the potential risk of some metals. Consequently, iron determination and speciation methods in food samples have been developed with high sensitivity. Most of methods are lack of sufficient sensitivity for iron speciation at $\mu\text{g/l}$ or sub- $\mu\text{g/l}$ levels. The high selectivity and sensitivity of atomic absorption methods overcome the problems of spectrophotometric methods for the speciation of iron. Solvent extraction and GFAAS methods were developed for analysis of iron in variety sample such as natural water, wastewater and solid samples, which are rapid extraction, simple tool and high sensitive when compare with spectrophotometric technique.