CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and Chemicals

2.1.1 Apparatus

1) Atomic absorption spectrometer, model AAnalyst 800 (Perkin-Elmer, U.S.A.)
2) THGA Graphite tube (Perkin-Elmer, U.S.A.)
3) HCL Lamp (Perkin-Elmer, U.S.A.)
4) Microwave digestion system, model Ethos Sel (Milestone Ethos, Italy)
5) Micropipette
   a) 0.5-10 μL, P series (Gilson Medical Electronics, France)
   b) 20-200 μL, P series (Gilson Medical Electronics, France)
   c) 100-1000 μL, P series (Gilson Medical Electronics, France)
6) Blender, model AY46R4 (Moulinex, China)
7) Shaker, (Memmert, Germany)
8) Stirrer, model RH basic 1 (Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
9) Magnetic bar (Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
10) Analytical balance, AB204-S series (Mettler Toledo, Switzerland)
11) pH meter model pH 713 (Metrohm, Switzerland)

12) Seive, mesh No. 52 (Endecotts (Filters) Limited, England)

13) Filter paper, No. 42 (Whatman, England)

14) Polyethylene (PE) bottles

2.1.2 Chemicals

1) Ammonium ferrous sulfate hexahydrate: $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (BDH, England)
2) Ammonium ferric sulfate dodecahydrate: $NH_4Fe(SO_4)_2 \cdot 12H_2O$ (BDH, England)
3) GFAAS mixed standard solution (Perkin-Elmer life and Analytical Sciences, USA)
4) 1-(2-Pyridylazo)-2-Naphthol (PAN): $C_{15}H_{11}N_3O$ (Carlo Erba, Italy)
5) Chloroform (AR grade, Merck, Germany)
6) Nitric acid, 65% (AR grade, Merck, Germany)
7) Hydrochloric acid, 37% (AR grade, Merck, Germany)
8) Ethanol, 95% (AR grade, Merck, Germany)
9) Glacial acetic acid (AnalaR, BDH, England)
10) Methyl isobutyl ketone (MIBK) (AR grade, Merck, Germany)
11) Argon gas, 99.999% (UHP grade TIG, Thailand)
12) Ultra purified water, resistivity 18.2 MΩ·cm at 25 °C (Millipore, USA)

2.2 Preparation Standard Solutions and Reagents

Ultrapurified water was used to prepare all solutions. All labware used for handling solution was cleaned with detergent solution, rinsed with tap water, soaked for at least 24 h in 10% nitric acid and then rinsed with ultrapurified water before use.

2.2.1 Standard solution of ferrous iron

Stock standard solution of 1000 ppm Fe(II) was prepared by dissolving 0.7143 g of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ in 50 ml of 0.1M HCl and made up to 100 ml with 0.1M HCl in volumetric flask.

2.2.2 Standard solution of ferric iron

Stock standard solution of 1000 ppm Fe(III) was prepared by dissolving 0.8690 g of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 50 ml of 0.1M HCl and made up to 100 ml with 0.1M HCl in volumetric flask.

2.2.3 1-(2-Pyridylazo)-2-Naphthol (PAN) solution

The PAN solution was prepared daily by dissolving 0.0500 g of PAN in 50 mL of ethanol.

2.2.4 Magnesium nitrate 1000 ppb

$Mg(NO_3)_2$ 1000 ppm was prepared by dissolving 0.0148 g $Mg(NO_3)_2$ in 100 ml ultrapurified water.

$Mg(NO_3)_2$ 1000 ppb was prepared by pipetting 1 ml of $Mg(NO_3)_2$ 1000 ppm into 100 ml volumetric flask and adjusting to mark with ultrapurified water.

2.2.5 Nitric acid solution

A 2% HNO_3 solution was prepared daily by adding 1.5 ml of concentration HNO_3 solution in ultrapurified water and adjusting to 250 ml with ultrapurified water in volumetric flask.

2.2.6 Acetic acid solution

A 1% CH_3COOH solution was prepared by adding 2.5 ml of CH_3COOH solution in ultrapurified water and adjusting to 250 ml with ultrapurified water in volumetric flask.

2.2.7 Hydrochloric acid solution

0.005M, 0.01M and 0.1M HCl were prepared by appropriate dilution of conc. HCl with water and adjusting to 500 ml in volumetric flask.

2.2.8 Preparation of the working standard solutions

The stock standard solution of iron (1000 ppb) was prepared by appropriate dilution of 20 ppm mixed standard iron with 2% HNO_3. This primary standard solution was used for preparation of working standard solutions.

Working standard solutions for calibration curve at concentrations of 10, 20, 60, 80, 100 ppb were prepared daily by transferring portions of 250, 500, 1500, 2000 and 2500 µl of stock solution into 10 ml volumetric flasks and added 375 µl of $Mg(NO_3)_2$ and adjusting to mark with 2 % HNO_3.

2.3 Sample Preparation

The rice samples were ground and homogenized with a blender and then sieved through a 52 mesh sieve. Samples were stored in PE bottles and kept in room temperature until required for analysis.

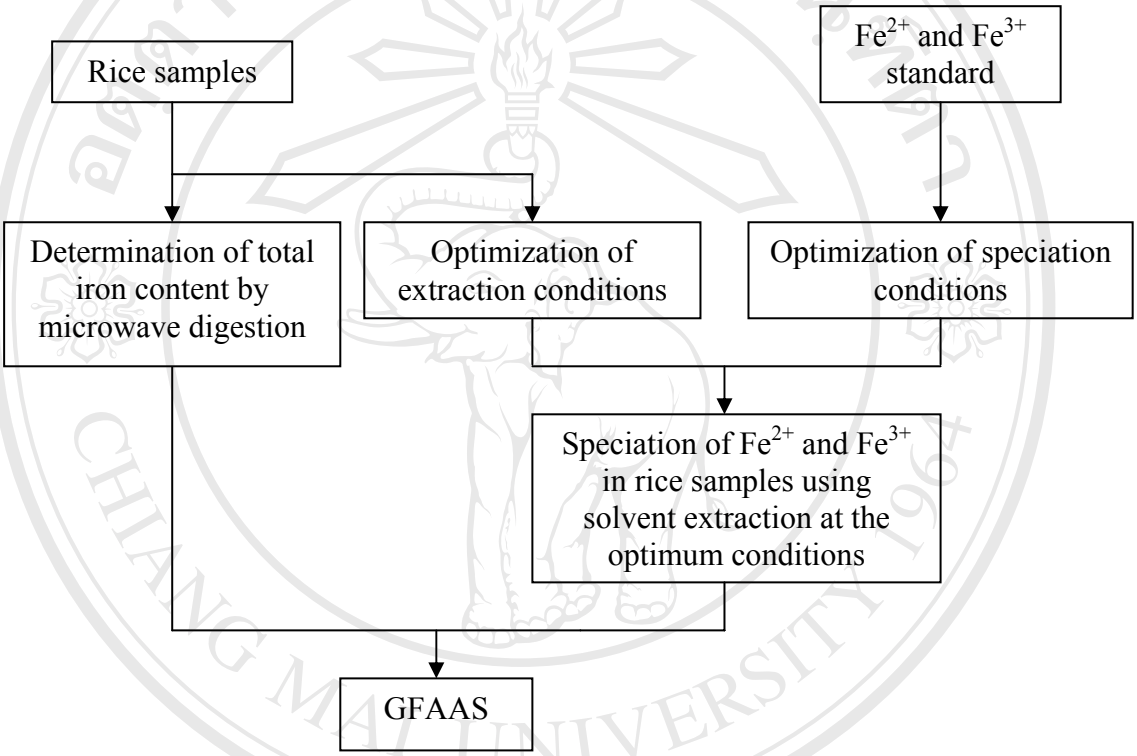

Figure 2.1 Summary of experimental procedures for the speciation of Fe^{2+} and Fe^{3+} in rice samples by solvent extraction technique and determine by GFAAS.

2.4 Analytical characteristics of the method

1) Linearity

In order to study the linear ranges, iron standard solutions at concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 ppb were examined.

2) Precision

The precision of the method and instrument were studied by analyzing 20 ppb standard solutions ten times. The relative standard deviation was calculated.

3) Detection limit

The detection limit of the method for determination of iron was determined by analyzing blank ten times.

4) Accuracy

Accuracy was calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample.

The extracted sample solutions was prepared by spiking 60 ppb and 60 ppb of Fe^{2+} and Fe^{3+} standard solutions, respectively to 25 ml of extracted sample solutions prior to extraction as described in **Figure 2.3**. The recovery was replicated three times and the results were calculated from the following equation.

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

2.5 Determination of total iron in rice samples [36]

Accurately 0.5 g of sample was weighted into a Teflon vessel and digested with 10 ml of conc. HNO_3 in microwave digestion system at 100 ^oC (500W) for 10 min and then 120 ^oC (1000W) for 120 min. After digestion, the clear solution were diluted to 25 ml with 0.2% HNO_3 and determined by GFAAS. Blank solutions were digested likewise. The operating conditions for microwave digestion system are shown in **Table 2.1**.

Table 2.1 Operating conditions for microwave digestion system

Step	Temp. (^oC)	Time (min.)	Power (Watt)
I	100	10	500
II	120	120	1000

2.6 Extraction method

For the sample preparation method, extractable iron in environment samples is extracted by solvent such as methyl isobutyl ketone (MIBK) [37-38], Chloroform ($CHCl_3$) [13], acetic acid (CH_3COOH) [39-40] and hydrochloric acid (HCl) [41], and. In addition, soluble iron sample is prepared by water [16]. In this work, seven kinds of solvent or solution including MIBK, $CHCl_3$, 1%CH_3COOH, 0.005M HCl, 0.01M HCl, 0.1M HCl and ultrapurified water were tested for the extraction process. The experimental procedures for extraction are shown in **Figure 2.2**.

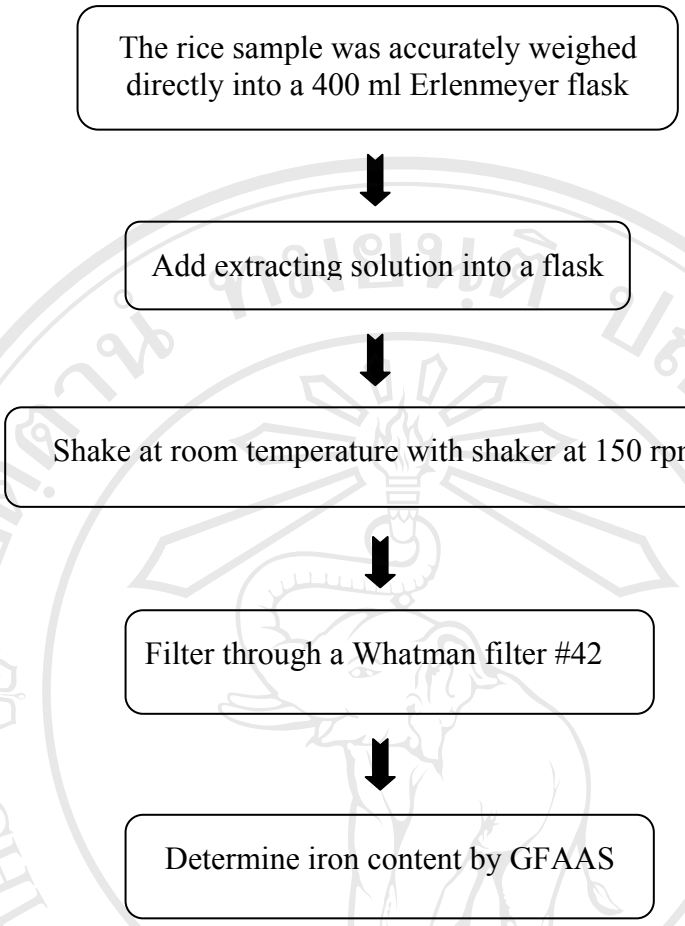

Figure 2.2 The experimental procedure for extraction.

2.7 Optimization of extraction method

The several parameters that influence on the extraction efficiency should be studied and optimized. Such factors including extraction solvent, weight of sample, volume of the extractant and extraction time. The extracted solutions were determined by graphite furnace atomic absorption spectrometry. The optimum conditions in this study were obtained by the maximum of iron condition.

2.7.1 Optimization of MIBK extraction method

The optimization of MIBK extraction method were studied by varying the ratio of rice weight per MIBK volume 1:25 and 1:50 g/ml and the extraction time from 15 to 60 min.

2.7.2 Optimization of chloroform extraction method

The optimization of chloroform extraction method were studied by varying the ratio of rice weight per chloroform volume 1:25 and 1:50 g/ml and the extraction time from 15 to 60 min.

2.7.3 Optimization of 1% acetic acid extraction method

The optimization of 1% acetic acid extraction method were studied by varying the ratio of rice weight per 1% acetic acid volume 1:25 and 1:50 g/ml and the extraction time from 15 to 60 min.

2.7.4 Optimization of 0.005M Hydrochloric acid extraction method

The optimization of 0.005M HCl extraction method were studied by varying the ratio of rice weight per 0.005M HCl volume 1:50 and 1:100 g/ml and the extraction time from 2 to 24 hours.

2.7.5 Optimization of 0.01M Hydrochloric acid extraction method

The optimization of 0.01M HCl extraction method were studied by varying the ratio of rice weight per 0.01M HCl volume 1:50 and 1:100 g/ml and the extraction time from 2 to 24 hours.

2.7.6 Optimization of 0.1M Hydrochloric acid extraction method

The optimization of 0.1M HCl extraction method were studied by varying the ratio of rice weight per 0.1M HCl volume 1:50 and 1:100 g/ml and the extraction time from 2 to 24 hours.

2.7.7 Optimization of water extraction method

The optimization of water extraction method were studied by varying the ratio of rice weight per water volume 1:50 and 1:100 g/ml and the extraction time from 2 to 24 hours.

2.8 Speciation Procedure [13]

The analytical steps were followed for separation procedure of Fe^{2+} and Fe^{2+} is shown in **Figure 2.3**.

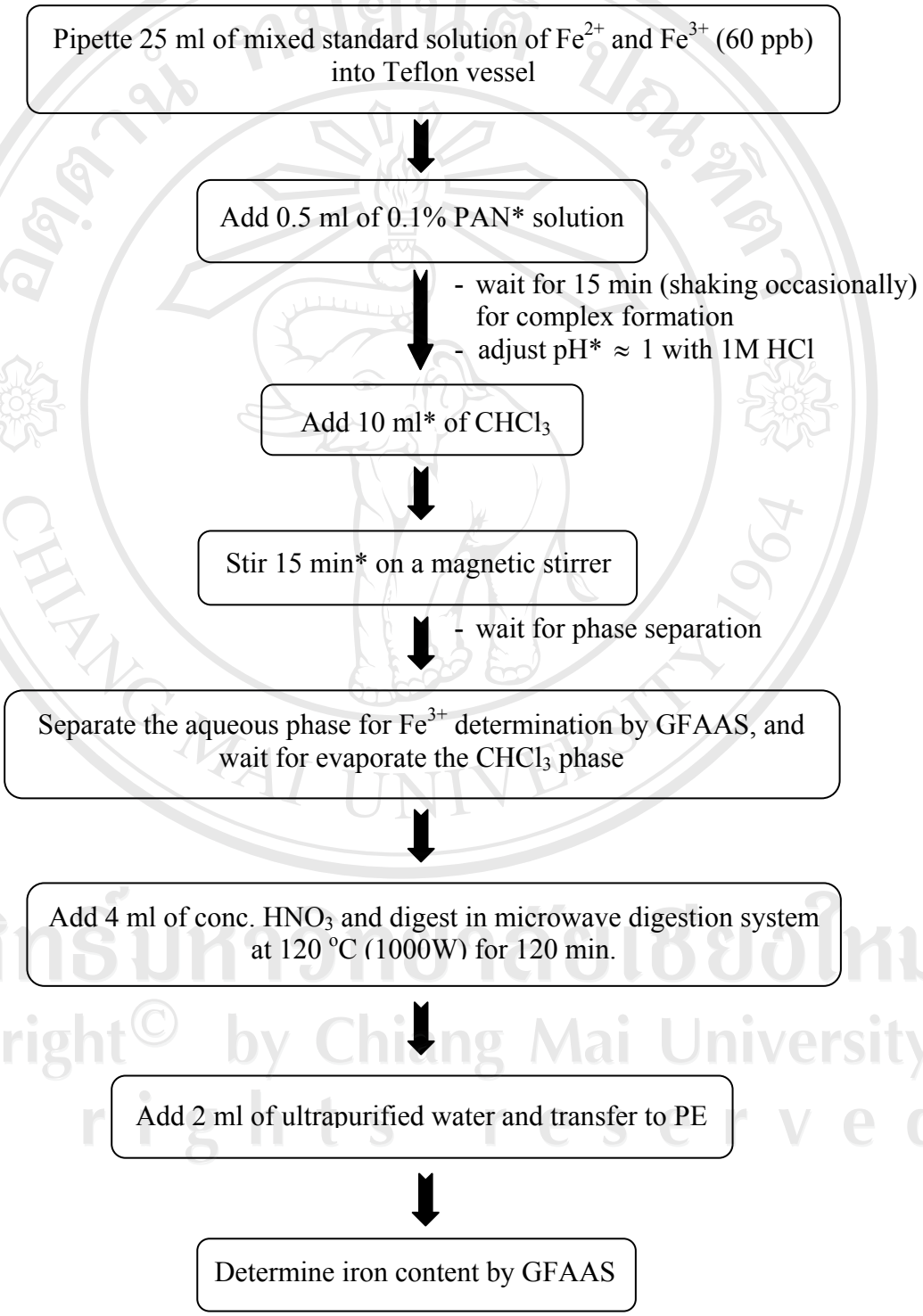

Figure 2.3 Scheme for separation procedure of Fe^{2+} and Fe^{3+}.

2.9 Optimization of speciation method

The optimized separation conditions in this study were defined as the conditions which were the maximum recovery for Fe^{2+} in chloroform phase and the maximum recovery for Fe^{3+} in aqueous phase were obtained The conditions for separation were optimized by using mixed standard solution of 60 ppb Fe^{2+} and Fe^{3+}. The following parameters were optimized: pH, the extraction time, the PAN amount and the chloroform volume are shown in **Table 2.2**.

Firstly, the optimization of pH was carried out in the range of 0.5-5.0 by using 1M HCl. Then, the description of procedure in **Figure 2.3** was applied. For the optimization of the extraction time was varied in the range of 5-30 min at the optimum pH and the other same conditions. Similarly, the optimization of PAN amounts in 25 ml of ethanol was varied in the range of 0.025-1.0 g at the optimum conditions of pH and extraction time. Finally, the chloroform volume was investigated in the range of 5-20 ml at the optimum conditions of the speciation method.

Table 2.2 The range of varied parameters for optimization of speciation method

Parameter	Range
pH	0.5-5
Extraction time (min)	5-30
% PAN (w/v)	0.1-0.5
Chloroform volume (ml)	5-20

2.10 Instrumentation

A Perkin Elmer AAnalyst 800 atomic absorption spectrometer equipped with THGA graphite furnace and with Zeeman effect background corrector was used. A Fe Lumina hallow cathode lamp was used as the resonance line source. For graphite furnace measurements, argon was used as inert gas. The operating parameters for working element were set as recommended by the manufacturer given in **Table 2.3** and **Table 2.4**. Perkin-Elmer pyrolytic-coated graphite tubes with a platform were used. Sample solutions were injected into the furnace using Perkin Elmer AS-800 autosampler. The injected volume of the sample was 20 µl. The $Mg(NO_3)_2$ were used as a metrix modifier. The signals were measured as peak areas. The range of the calibration standards for iron was 10-100 ppb.

Table 2.3 Instrument settings and analytical conditions

Parameter	Condition
Wavelength (nm)	248.3
Slit width (nm)	0.2
Argon flow (ml/min)	250
Atomization site	Pyro/Platform
Reading time (min)	5
Pretreatment temperature (^{o}C)	1400
Atomization temperature (^{o}C)	2100

Table 2.4 Furnace heating program for iron determination

Step	Temperature (^{o}C)	Ramp Time (s)	Hold Time (s)	Gas Flow (ml/min)
Dry 1	110	1	20	250
Dry 2	130	5	30	250
Pyrolysis	1400	10	20	250
Atomize	2100	0	5	0
Clean-out	2400	1	2	250

2.11 Determination of Fe^{2+} and Fe^{2+} in rice bran samples

Rice bran samples were selected for the determined of Fe^{2+} and Fe^{3+} was carried out by using the optimum conditions of extraction method and speciation procedure. The experimental procedures for extraction and speciation are shown in **Figure 2.4**.

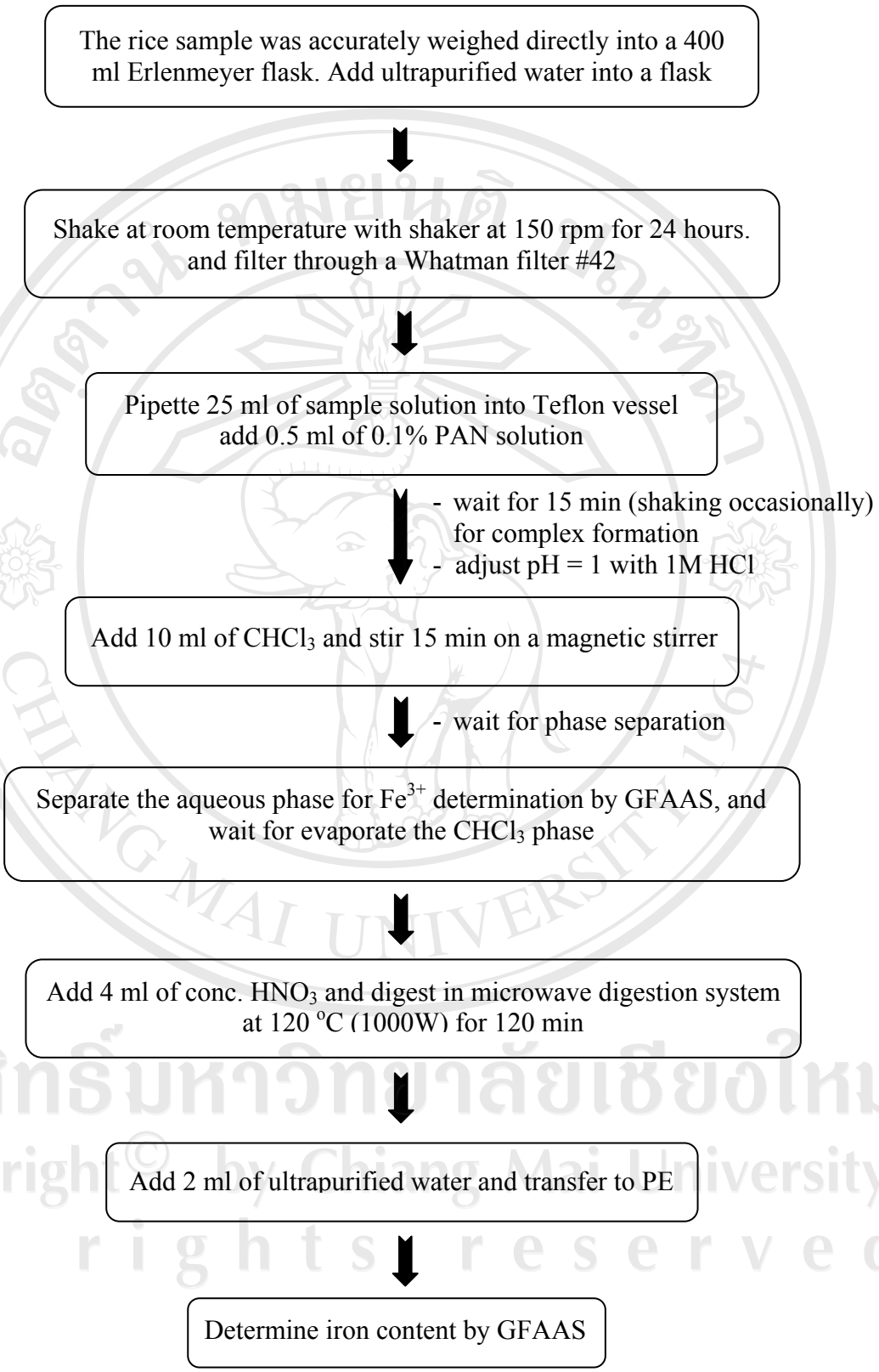

Figure 2.4 Scheme for separation procedure of Fe^{2+} and Fe^{3+} in rice bran samples.

2.12 Rice and rice bran samples

The rice samples were collected from various sites in Chiang Mai, Thailand. And rice bran samples were collected from Tung Kula Ronghai (the area covers the vicinities of five provinces: Surin, Roi Et, Maha Sarakham, Buriram, and Yasothon), Thailand as described in **Table 2.5**.

Table 2.5 Rice and rice bran samples from various areas

Sample	Type of sample	Sampling site
Rice-1	Brown rice	Amphoe Doi Saket
Rice-2	Brown rice	Amphoe Sa Meong
Rice-3	Brown rice	Doi Kham
Rice-4	Black rice	Faculty of Agricultural, Chiang Mai University
Rice-5	Black rice	Doi Kham
Rice-6	Red rice	Faculty of Agricultural, Chiang Mai University
Rice bran-1	Black rice bran	Tung Kula Ronghai
Rice bran-2	Black rice bran	Tung Kula Ronghai
Rice bran-3	Black rice bran	Tung Kula Ronghai
Rice bran-4	Black rice bran	Tung Kula Ronghai
Rice bran-5	Black rice bran	Tung Kula Ronghai