CHAPTER 2

METHODOLOGY

2.1 Chemical and material

All chemicals used in this work were analytical grade and prepared in double distilled water (DDW). All plasticwares and glasswares were pre cleaned by soaking in 6% (v/v) nitric acid (HNO_3) for 24 hours and then were rinsed with DDW. The standard arsenate stock solution and lists of reagent used are given in Table A-1 in Appendix A. The chemical preparation details for reagents, stock and working solution and arsenic solutions for calibration curves are given in Appendix B.

2.2 Instruments

Atomic absorption spectrophotometers (AAS), Perkin Elmer model 5000 (Figure 2-1) and Perkin Elmer AAnalyst 800 (Figure 2-2), coupled with an automatic flow injection systems were used to measure arsenic concentration in the solutions. The conditions for use of this instrument are given in Table A-2 and Table A-3 in Appendix A, respectively.

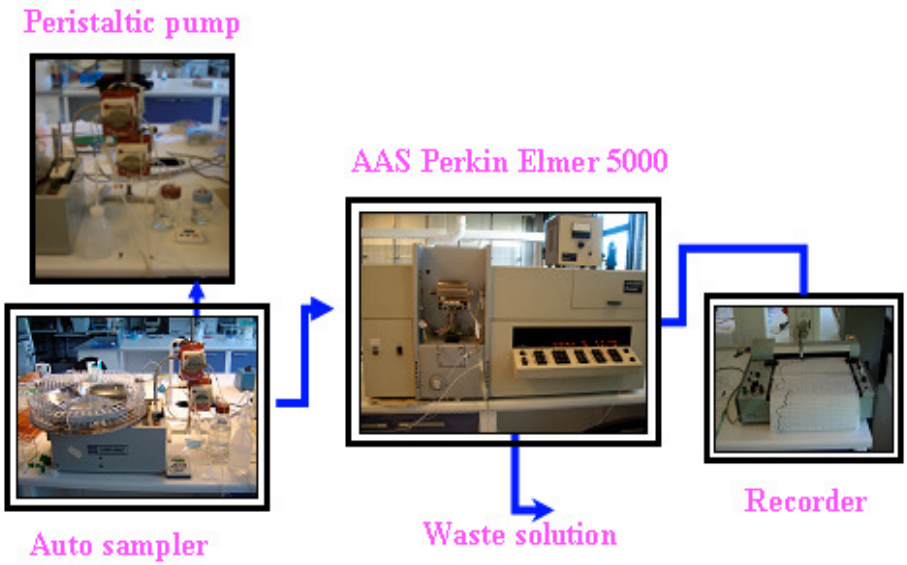


Figure 2- 1 Perkin Elmer model 5000 coupled with Flow injection system

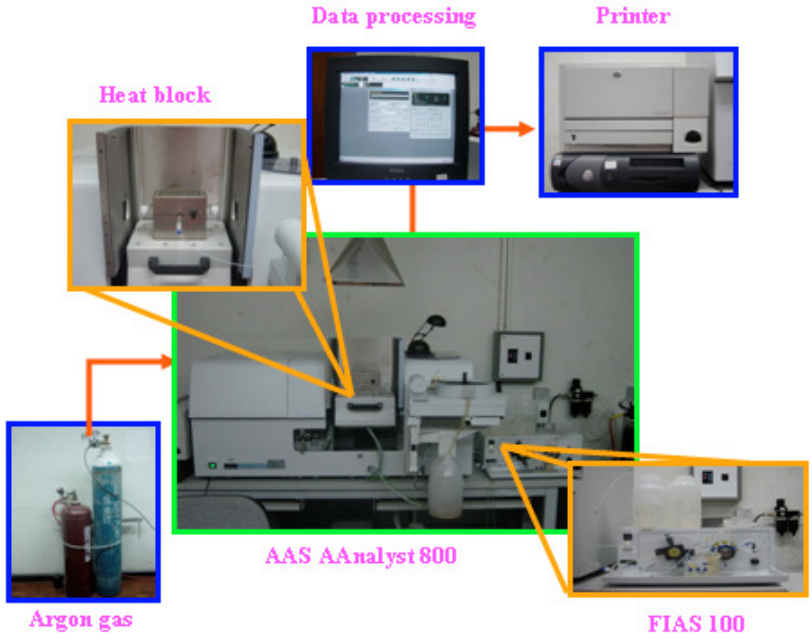


Figure 2- 2 Perkin Elmer model AAnalyst 800 coupled with Flow injection analysis system 100 (FIAS100)

2.3 Hydride Generation Atomic Absorption Spectrophotometry (HGAAS)

The hydride generation (HG) technique combined with atomic absorption spectrophotometer (AAS) was used to determine total arsenic concentration in soil and edible plant samples. The complete of arsine generation can be easily obtained from As(III), therefore, total inorganic arsenic in solution needs to be converted to As(III) before the analysis (pre-reduction step). The most popular pre-reducing agent is a combination of potassium iodide (KI) and ascorbic acid, in order to prevent the oxidation of iodide to triiodide by air, moreover $NaBH_4$ was also found to reduce As(V) to As(III) at the high concentration. Other reagents used for reduction of As(V) are mercaptoacetic acid and L-cysteine which was found also to reduce interferences and increase the sensitivity (Hung *et al*., 2004).

In this study, inorganic arsenic species present in the solution were reduced and generated to arsine using 3% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH for AAS Perkin Elmer model 5000. On the other hand, inorganic arsenic species were converted to arsine by 0.3% (w/v) $NaBH_4$ in 0.1% (w/v) NaOH after being reduced with potassium iodide mixed with ascorbic acid (FIAS 100 coupled with AAnalyst 800). The chemical reactions of an arsine generation from the $NaBH_4$ are shown in Equations (2-1) to (2-4).

$$NaBH_4 \rightarrow Na^+ + BH_4^- \qquad (2-1)$$

$$2BH_4^- \rightarrow B_2H_6 + H_2 + 2e^- \qquad (2-2)$$

$$As^{3+} + 3H^+ + 6e^- \rightarrow AsH_3 \qquad (2-3)$$

$$6BH_4^- + As^{3+} + 3H^+ \rightarrow 3B_2H_6 + 3H_2 + AsH_3 \qquad (2-3)$$

$$B_2H_6 + 6H_2O \rightarrow 2H_3BO_3 + 6H_2 \qquad (2-4)$$

Chemical reaction of an arsine generation

Source: Amornsit and Petsom, 1992

After the follwing of the reaction the arsine gas was purged with carrier gas (nitrogen gas for Perkin Elmer model 5000 and argon gas for FIAS 100

coupled with AAnalyst 800) into an electrically heated quartz cell (900ºC) where they were atomized. Analytical signals were recorded by a computer linked to the AAS that was coupled with peak high (Perkin Elmer model 5000) and peak area measurements (AAnalyst 800). Optimization of FIAS 100-Perkin Elmer AAS AAnalyst 800- is described in next section.

2.4 Optimization of the analytical method

The optimization was performed by varying one parameter, while other parameters were kept constant. The optimum values were selected. All experiments were carried out using 4 $\mu g\ L^{-1}$ arsenate stock solution. The following parameters for FIAS 100 coupled with AAnalyst 800 were performed including:

2.4.1 Carrier gas (Ar) flow rate

Atomization temperature was fixed at 900 °C. A 0.5% (w/v) $NaBH_4$, 10% (v/v) HCl, 5% (w/v) KI mixed with 5% (w/v) ascorbic acid were used as agent solution and allowing 45 minutes for the reduction time (recommended condition in FIAS 100 manual). The optimum carrier gas (argon gas) flow rate was investigated at 40, 45, 50 and 75 ml min^{-1}. Three replicates were performed at each flow rate.

2.4.2 Effect of $NaBH_4$ concentration

The effect of the $NaBH_4$ concentration was investigated at 0.1, 0.3, 0.5 and 0.7 % (w/v). The optimum carrier gas flow rate taken from 2.4.1 was used. Three replicates were performed for each concentration.

2.4.3 Effect of HCl concentration

The effect of HCl concentration was investigated at 1, 5, 10, 15 and 20 % (v/v). The optimum carrier gas flow rate (from 2.4.1) and $NaBH_4$ concentration (from 2.4.2) were used, other parameters were the same as 2.4.1. Three replicates were performed.

2.4.4 Effect of KI / Ascorbic acid concentration

All optimal conditions from 2.4.1, 2.4.2, and 2.4.3 were used in this experiment. The effect of reducing solution concentration (KI + ascorbic acid) were

studied at 1+1, 3+3, 5+5, 7+7, 9+9 % (w/v). Three replicates were conducted for each mixture.

2.4.5 Effect of reduction time

The effect of reduction time after all above conditions was optimized. The reducing times were investigated at 0, 15, 30, 45, 60 and 75 min and three replicates were performed for each experiment.

2.4.6 Optimum of atomization temperature

The atomization temperature should be high enough to complete the atomization of arsine, therefore, the atomization temperature at 700, 750, 800, 850, and 900 °C were investigated. Three replicates were performed for each temperature.

2.5 Sampling site and sample preparation

According to previous works in Ronphibun Sub-district, (i.e. Na Chiengmai, 1991 and Rakwong, 1999), it was concluded that Villages No. 1, 2, 12 and 13 are high risk areas, whereas Villages No. 8, 9, 11 and 14 are low risk areas. In this study soil and edible samples were collected from these villages. Five soil samples were collected from each village. Edible plant samples growing at the sample site were also collected. Forty soil samples and 121 edible plant samples were collected during March 2004 (Figure 2-3). The names of village used for this study are shown in Table 2-1.

Table 2- 1 The Village names of the sampling area in Ronphibun Sub-district, Nakhorn Si Thammarat

Village No. (Moo)	Village Name
1	Ban Hu dan
2	Ban Ronna
8	Ban Ton gor
9	Ban Moung Ngam
11	Ban Hoy kean
12	Ban Talard ron
13	Ban Salakheleg
14	Ban Na phoe

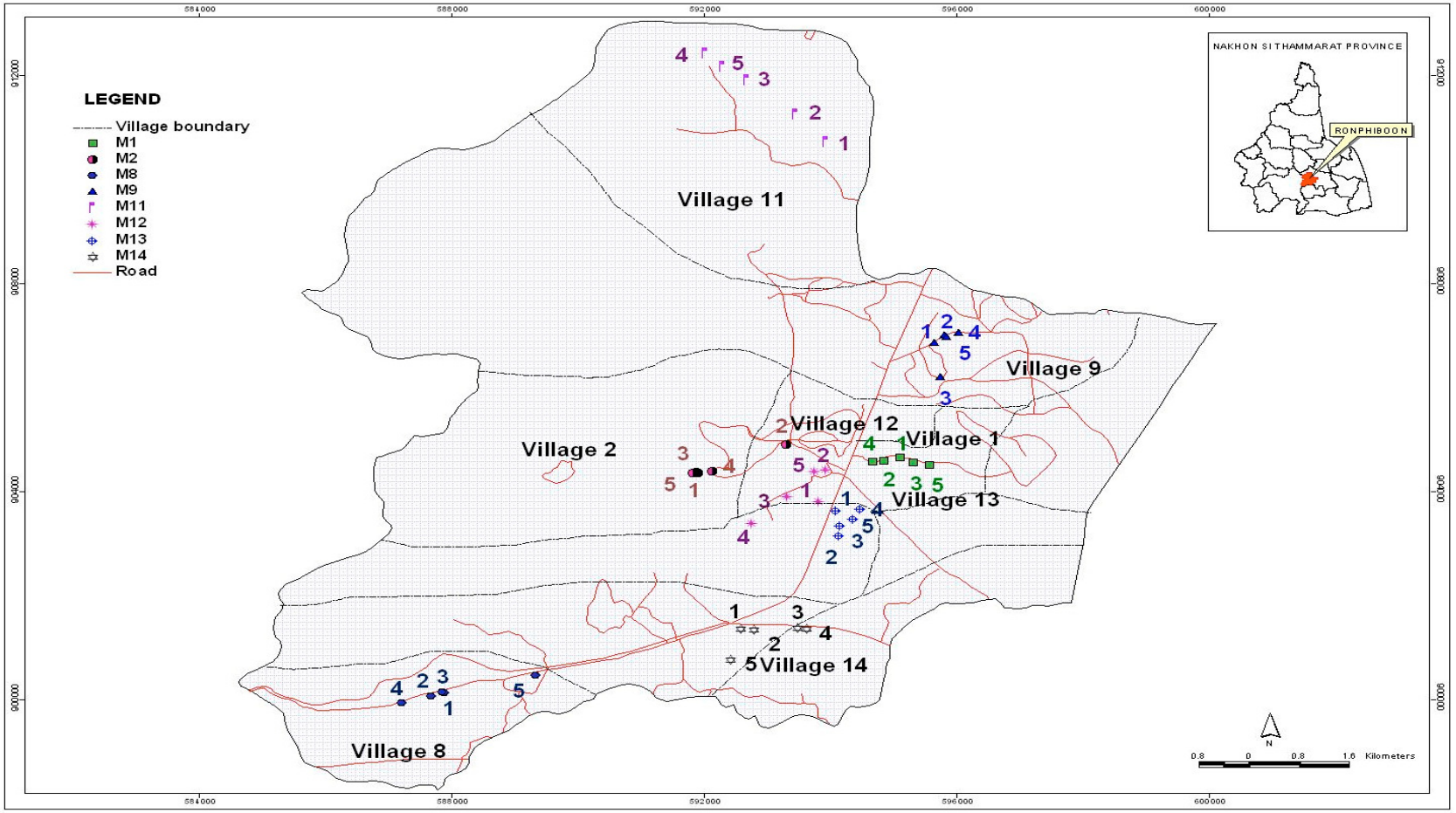


Figure 2- 3 The sampling location (see details of each station in Table A-4 in Appendix A)

2.5.1 Soil sampling and preparation

Five topsoil samples were collected from each village. One kilogram composite sample of topsoil (around 15 cm depth) was collected. The sampling protocol is shown in Figure 2-4. Five sub-samples in square foot (12x12 inch) of each station were collected and mixed to obtain a composite sample (Tan, 2005).

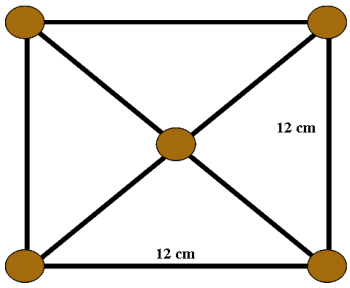


Figure 2- 4 Compositing sampling protocol

After collection, soil samples were dried in an oven at 55-60^{o}C for 12 hours or until dryness. A portion approx. 125 g. of the sample was passed through a 2-mm sieved to remove stones, debris and plant materials. Each sieved sample was then ground using an agate mortar. The fine samples were kept in plastic bags until analysis.

2.5.2 Plant sampling and preparation

All kinds of edible plants (illustration in Figure 2-5) were collected from the same location as the soil samples. The plant samples were identified using the key in *Thai plant names* (Smitinande, 2001). After sampling, the plants were washed with tap water to get rid of dirt, then rinsed with nanopure water (>18 mΩ) prior to be dried in oven until constant weight at 50±2 ^{o}C (approx. 4-6 days). Individual sample was cut into small pieces using an electrical blender and kept in a plastic bag.

Figure 2- 5 Edible plants collected from Villages No. 1, 2, 8, 9, 11, 13 and 14 grown in Ronphibun Sub-district

2.6 Comparison of the extraction methods

In order to gain the optimum results, two extraction methods (autoclave extraction and hot plate extraction) were compared.

2.6.1 Autoclave extraction method

Two grams of soil sample was accurately weighed and placed into a Pyrex bottle. Twenty ml of 7M HNO_3 was added. Extraction was performed in an autoclave oven (120°C, 2 atm) for 30 minutes. After cooling, the solution was filtered through a 0.45 μm (Whatman) membrane filter. The final volume of extraction solution was adjusted to 50 ml with DDW.

2.6.2 Hot plate extraction method

Two grams of soil sample was accurately weighed and placed into a 125 ml Erlenmeyer flask. Twenty ml of (1:1, v/v) HNO_3:H_2O was added and covered the flask with a watch glass. The mixture was brought to a slow boil on a hot plate at 85±5°C until extraction was completed (about 8 hours) as shown by a light-color or a clear solution. To prevent samples from drying out during extraction, a small volume of 1:1 (v/v) HNO_3 was added if necessary. After cooling, the extraction solution was adjusted to a final volume of 50 ml with DDW.

2.7 Samples Extraction

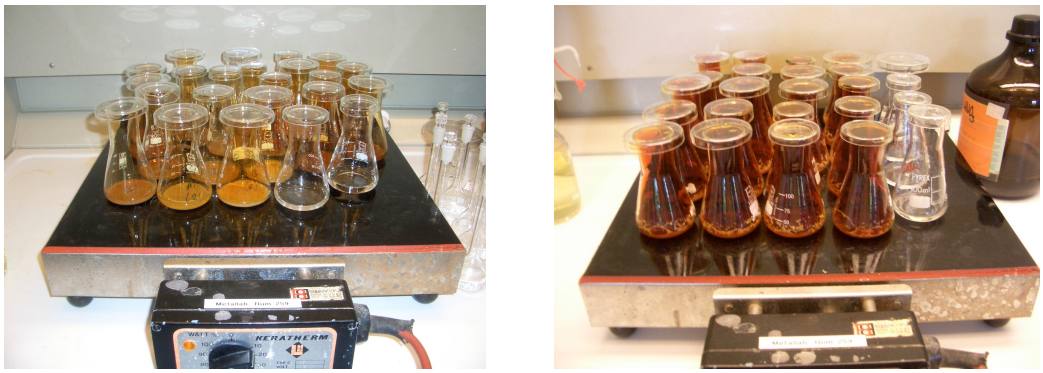
2.7.1 Soil extraction

The hot plate extraction method (described in section 2.6.2) was chosen according to the results as shown in chapter 3, section 3.1 (Figure 2-6A). All the samples and all the analysis were duplicated.

2.7.2 Plant extraction

The hot plate extraction method was also employed for plant samples preparation. However, the plant tissue required stronger oxidizing reaction to decompose organic matter. Therefore, a small amount of conc. $HClO_4$ was added during extraction step to plant samples. One gram of the dried plant sample was weighed accurately and placed into the 125 ml Erlenmeyer flask. Ten ml of conc.

HNO_3, was added following by 1 ml conc. $HClO_4$. The flask was then covered with a watch glass and brought to a slow boil on a hot plate at 80±5°C (Figure 2-6B). Heating was continued until the solution was clear. To prevent samples from dryness, 5 ml of conc. HNO_3 and 0.5 ml of conc. $HClO_4$ were added if necessary. After cooling, the sample was adjusted to a final volume of 10 ml with DDW. All the samples and all the analysis were duplicated.



A B

Figure 2- 6 Samples extraction (A: soil extraction. and B: plant extraction)

2.8 Determination of total arsenic in soils and edible plants

The HGAAS analyses of arsenic were performed at both Technical University of Denmark (DTU) in Denmark and Prince of Songkla University (PSU).

The equipment used at DTU was the Perkin Elmer Model 5000 AAS. Thirty five soil samples and sixty nine plant samples were performed. All instrumental conditions and reagents followed a laboratory protocol developed by the Department of Environmental & Resources, DTU.

FIAS 100 coupled with AAnalyst 800 was used to analyze five soil samples and fifty-two plant samples at the Department of Chemistry, Faculty of Science, Prince of Songkla University. Potassium Iodide (KI) mixed with ascorbic acid was used as reducing agents to reduce As(V) to As(III) prior to generate hydride. The parameters were set following the results from section 2.4

2.9 Standard addition method

The extractants from the natural samples always contain various matrices. Standard addition is usually used to compensate matrix interference in the samples. Arsenic from stock standard solution was added into the soil sample (taken from $M_7B_{79/1}$) to give an additional arsenic concentration of 1, 2, 4 and 8 μgL^{-1}, and was added into *Alpinia* sp. (taken from M_8 B_{93}) to give an additional arsenic concentration of 1, 2 and 4 μgL^{-1}. Three replicates were done at each concentration.

2.10 Method of validation

2.10.1 Detection limit (DL)

The detection limit (IUPAC definition) is expressed as the smallest concentration that can be detected with a certainty of more than 95%. It is defined as the analyte concentration yielding a response k folds higher than the standard deviation of the blank (s_b) (k is defined as the confidence factor), the calculation of detection limit is given in Equation 2-5 and 2-6 (Skoog *et al*., 2004)

$$DL = \frac{ks_b}{m} \tag{2-5}$$

$$s = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{(n-1)}} \tag{2-6}$$

S = standard deviation

n = total number of values

x_i = each individual value used to calculate mean

$\bar{x}$ = mean of n values

Where m is the slope of calibration curve, and factor k is chosen to be at 3 in order to gain a 98.3 % confidence level (Skoog *et al*., 2004). It is normal to assume that the results of a blank and the sample will follow a normal distribution, and also assumed that the standard deviation from the blank and sample are the same. In this work, DL of IAS 100 coupled with AAnalyst 800 was calculated by using Equation 2-5 (when s_b value was obtained from 10 measurements of reagent blank signal).

However, for Perkin Elmer model 5000, the response signal from reagent blank and the base line signal (background = signal) of recorder were difficult to distinguish. Therefore, lowest concentration (5 μg L^{-1}) in the working curve was used to calculate instead of reagent blank.

2.10.2 Precision

Precision is the measure of the degree of an analytical method under the same conditions. Normally it is always expressed as a percentage of the relative standard deviation (%RSD) for a statically significant number of samples. The calculation of %RSD is given Equations 2-6 and 2-7 (Skoog *et al.,* 2004):

$$\%\,RSD = \frac{s}{\bar{x}} \times 100\% \qquad (2\text{-}7)$$

Extractants of soil from $B_{204}M_{14}$ and *Curcuma Longa* from M_2B_{237} were repeated 10 times to investigate the precision of the method.

2.10.3 Accuracy

To ensure that the analytical concentration is accurate, the measured value should agree with the accepted value of certified reference material (CRM). Normally, the accuracy value is expressed as the relative percent error term. The following calculation of relative percent error is given by Equation 2-8 (Skoog *et al.,* 2004):

$$\%\,Error = \frac{Measured\ value - Certified\ value}{Certified\ value}\ x100 \qquad (2\text{-}8)$$

The accuracy from this research was studied by extracting PACS-2, a CRM for sediment from the National Research Council of Canada, following the same method as used for soil extraction and determination (three replicates). The difference in values between the measured value and certified value were compared and the relative percent error was also calculated.

2.10.4 Recovery

The terms recovery (*R*) is used to indicate the yield of an analyte in a pre-concentration or extraction stage in an analytical method. Actually, the recovery

value is presented as a percent recovery (*%R*) and it can be calculated from the equation given Equation 2-9 (Rubinson, 1987).

$$Percent\ recovery = \frac{Measured\ value}{Certified\ value} x\ 100 \qquad (2\text{-}9)$$

To investigate of soil samples, 5, 10 and 20 µg arsenic were spiked into soil samples extractants before analyzing. Whereas, 0.1, 0.2 and 0.4 µg arsenic were used for plant samples. The spiked samples were left overnight prior to analysis. Three replicates were performed at each concentration.

2.10.5 Linear dynamic range

The linear dynamic range is the range of concentration that can be obtained from a linear calibration curve. Usually, a 5% deviation from the linearity is considered as the upper limit. The deviation from the linearity is usually found at the high concentrations due to non-ideal detector responses or chemical effects (Skoog *et al.,* 2004). The analysis of arsenic standards with concentrations in the range of 0-50 µg L^{-1} was performed for the linear dynamic range by using IAS 100 coupled with AAnalyst 800.