2. EXPERIMENTAL

2.1 Apparatus

2.1.1 Double-beam ultraviolet-visible spectrophotometer, Shimadzu Model UV-265, manufactured by Shimadzu Corporation, Japan.

2.1.2 Vacuum manifold, Baker column processing system Model Baker SPE-12G, manufactured by J.T.Baker, U.S.A.

2.1.3 Vacuum pump, Air cadet vacuum/pressure station Model 7559-60, manufactured by Cole-Parmer Instrument Company, U.S.A.

2.1.4 Buchner filter funnels and suction flask.

2.1.5 Autopipette, Socorex adjustable 20-200 ul, manufactured by Socorex, Switzerland.

2.1.6 Cuvette, Pye-unicam, 1 cm matched quartz cell cuvette.

2.2 Chemicals and Materials

2.2.1 Methanol, absolute, J.T.Baker, U.S.A.

2.2.2 Hydrochloric acid, 37% AR grade, Merck, Germany.

2.2.3 Ammonia solution, 25% AR grade, BDH, England.

2.2.4.Sodium dithionite, AR grade, Merck, Germany.

2.2.5 Sodium hydroxide pellet, Merck, Germany.

2.2.6 pH paper, Riedel-de Haen indicator strip with range 0-14.

2.2.7 SPE column, Bakerbond spe-cyano, 6 ml disposable extraction column, J.T.Baker, U.S.A.

2.2.8 Filter paper, Whatman filter paper no.1 and 6.

2.2.9 Paraquat dichloride, 100% w/w. I.C.I. Agrochemicals, England.

2.2.10 Distilled water.

2.3 Preparation of Standard Solutions

2.3.1 Stock standard solution of paraquat, 250 ug/ml.

A 0.0864 g amount of pure paraquat dichloride was dissolved in distilled water and the volume was adjusted to 250 ml. The solution was kept in a dark plastic container, stored in a refrigerator and equilibrated at room temperature before use. Due to its hygroscopic property, paraquat salt was dried at 100^0c for 5 hours and cooled in a desiccator before use[39].

2.3.2 Working standard solution of paraquat, 100 ug/ml, for recovery test.

A 20 ml volume of the stock solution was transferred into a 50 ml volumetric flask and added with distilled water to the mark. It was then kept in a dark plastic container, stored under refrigeration and equilibrated at room temperature before use.

2.3.3 Working standard solutions for constructing the calibration curves

Three sets of the standard paraquat solution were prepared by dilution of the stock solution with the mixture of 1.5M hydrochloric acid and ammonia solution (4:1 v/v).

2.3.3.1 Working standard solutions of low range of paraquat concentration (0.025, 0.05, 0.1 and 0.2 ug/ml)

A 20 ul volume of the stock solution were transferred into 25,50 and 100 ml volumetric flasks and each of the solution was added up to the mark to achieve the concentration of the three last working standard solutions. The 0.05 ug/ml working standard solution was then diluted one fold to yield the 0.025 ug/ml working standard solution.

2.3.3.2 Working standard solutions of medium range of paraquat concentration (0.5, 1, 1.5 and 2 ug/ml)

These solutions were obtained by pipetting 50, 100, 150 and 200 ul of the stock solution into four 25 ml volumetric flasks separately and each solution was added with the mixture solution to the mark.

2.3.3.3 Working standard solutions of high range of paraquat concentration (2, 4, 6 and 8 ug/ml)

These solutions were obtained by pipetting 200, 400, 600 and 800 ul of the stock solution into four 25 ml volumetric flasks and performing the same procedure as described in Section 2.3.3.2.

2.4 Preparation of Reagents

2.4.1 Sodium hydroxide, 0.5M.

2.4.2 Hydrochloric acid, 1.5M.

2.4.3 Sodium dithionite 0.2% in 0.5M sodium hydroxide.

A 0.1 g amount of sodium dithionite was dissolved in 50 ml of 0.5M sodium hydroxide and the solution was ensured to be well mixed. This freshly prepared solution was normally used within half an hour after preparation[39].

2.5 Spectrophotometric Conditions

The spectrophotometric conditions employed include the following.

Photometric mode : Abs.

Scan speed : Fast

Scale : 20 nm/cm

Photometric range:Low = 0 Abs. High =0.05, 0.5, 2 Abs.

Wavelength range : Start = 430 nm End = 340 nm

Light source change : 360 nm

Slit : 2 nm

2.6 Study Site

This research was carried out at a longan *(Dimocarpus longan Lour.)* plantation at **Ban Sop Pao, Muang District, Lamphun Province.** It was located at the road side about 30 km from Chiang Mai along the old Chiang Mai-Lamphun road as shown in Figure 2.1.

Two trials labelled V and L of three replicate plots of 4m x 5m size were designed for each of the two treatment types, the farmer's practice and the recommended practice, as shown in Figure 2.2. The areas contained grassy weeds such as *Paspalum conjugatum Berg.* and *Panicum repens.* which were mostly about 30-50 cm high and in the flowering stage. The weeds were slightly dense in the northern part of the site, becoming more dense towards the middle part and gradually declining towards the south and then very dense again at the southern end of the study site as shown in Figure 2.3. The

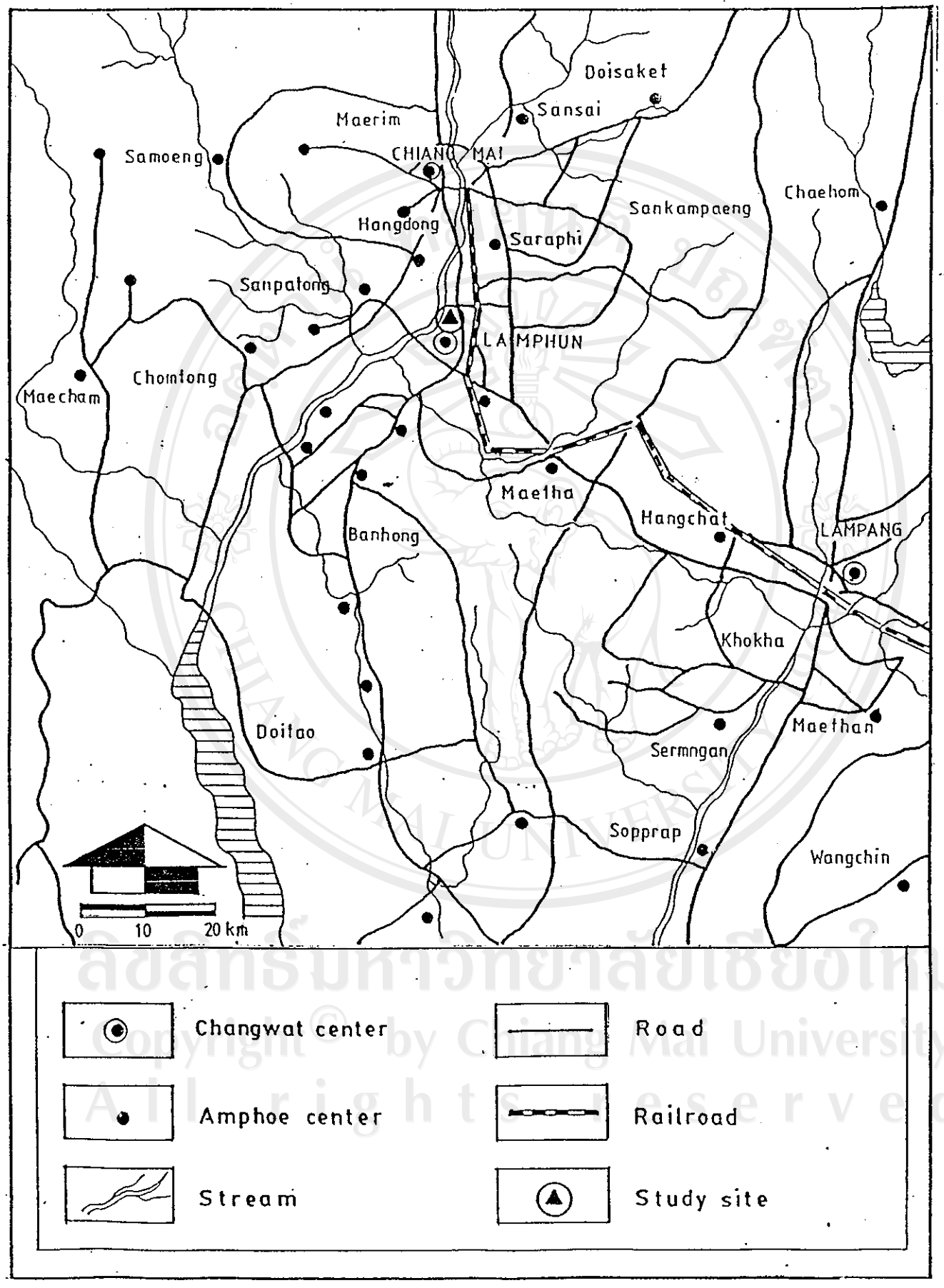

Figure 2.1 Map showing location of the study site.

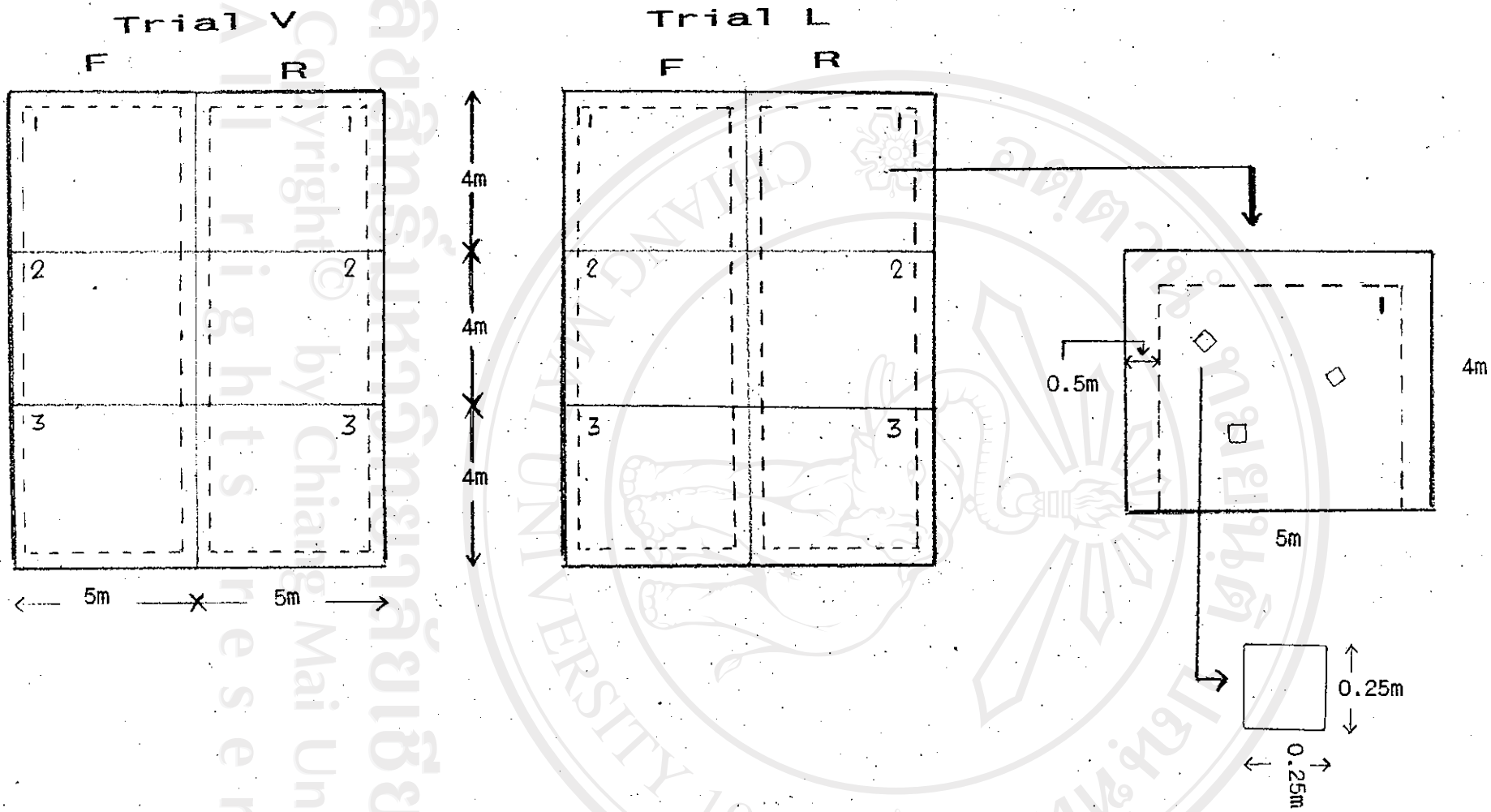

Figure 2.2 Diagram of the plots and zones of collecting weed samples.

Figure 2.3 Photograph of the study site.

study at this site was carried out during October 26, 1993 - November 4, 1993. The microclimate during the study period were also obtained from the nearest meteorological station.

2.7 Application of Paraquat

The spray solution of paraquat in this study was of two levels of dose. The farmer's practice was prepared by diluting 200 ml of the **Gramoxone 27.6% w/v SL**, a product of I.C.I. Asiatic (Agriculture) Company Ltd. containing paraquat dichloride 276 g/l or paraquat ion 200 g/l, with 20 liters of water. The agricultural officer's recommended practice was prepared by diluting 28ml of Gramoxone with 7 litres of water. The application of paraquat by both the farmer and the agricultural officer at the two trials at the study site was achieved using a knapsack equipped with the same type of hollow cone nozzle at 10.00 am on 27 October, 1993.

2.8 Sample Collection

Weeds above the ground level were collected randomly by means of three frames of 0.5m x 0.5m size at the collection zone from each plot of the two trials to represent weed samples for each of the individual plots. The sample collection just described was performed one day before the application and at the interval of times 0, 1, 2, 3 and 8 days after application. The collected weed samples were kept in separate labelled plastic bags before being kept in dark plastic bags. All the samples were transported to the laboratory of the Chemistry Department of Faculty of Science,

Chiang Mai University, for subsequent analyses.

2.9 Sample Preparation

2.9.1 Weed sample preparation

The weed samples were cut into small pieces (3-5 cm). Two replicates of 50 g each (sample A and B) were separately soaked in 600 ml volume of distilled water for 10 mins. They were then filtered through Whatman filter paper No. 1 and 6. The volumes of the filtrates were measured. The filtrates were kept in plastic bottles at room temperature at about 20^0C.

2.9.2 Solid phase extraction

2.9.2.1 Column conditioning

The cyano-spe columns were rinsed well with 10 ml of methanol, followed by 10 ml of distilled water and kept wet before use.

2.9.2.2 Sample extraction

The filtrates had been adjusted to yield pH 7.5 before they were passed through the column by using water aspiration at the flow rate about 1-2 ml/mins.

2.9.2.3 Column wash

The columns were connected to the vacuum manifold employed and washed with 10 ml of distilled water. The columns were then dried by means of an air pump suction for 5 minutes.

2.9.2.4 Sample elution

A 4 ml volume of 1.5M hydrochloric acid was used

to elute paraquat in each column gravitationally and the eluate was collected in a 5 ml volumetric flask. After no more drops of the eluate were observed from the column, air suction was applied to draw any remaining solvent from the column into the volumetric flask. A milliliter volume of ammonia solution was added to each of the collected eluate. The solution was well mixed and when it was necessary the mixture of 1.5M hydrochloric acid and ammonia solution (4:1 v/v) was added to make up the volume to 5 ml. Each of the columns was eluted twice. The cyano-spe sample preparation was shown in Figure 2.4.

2.10 Sample Analysis

The eluate was reduced by 1 ml of freshly prepared 0.2% sodium dithionite in 0.5M sodium hydroxide, gently mixed and then its absorption was immediately scanned from 430-340 nm. A baseline was drawn as a tangent to the curve from the valleys for each spectrum and the height of the peak was measured above the baseline at 396 nm, as shown in Figure 2.5[39]. Quantitation of paraquat in each sample was achieved by the external standard method whereby the standard calibration curve was obtained under the identical procedure. Amounts of paraquat were expressed as (1) milligram paraquat per kilogram based on the dry weight of weed samples and (2) gram of paraquat per hectare.

The whole analytical procedure and the analysis of paraquat can be summarized as shown in Figures 2.6 and 2.7.

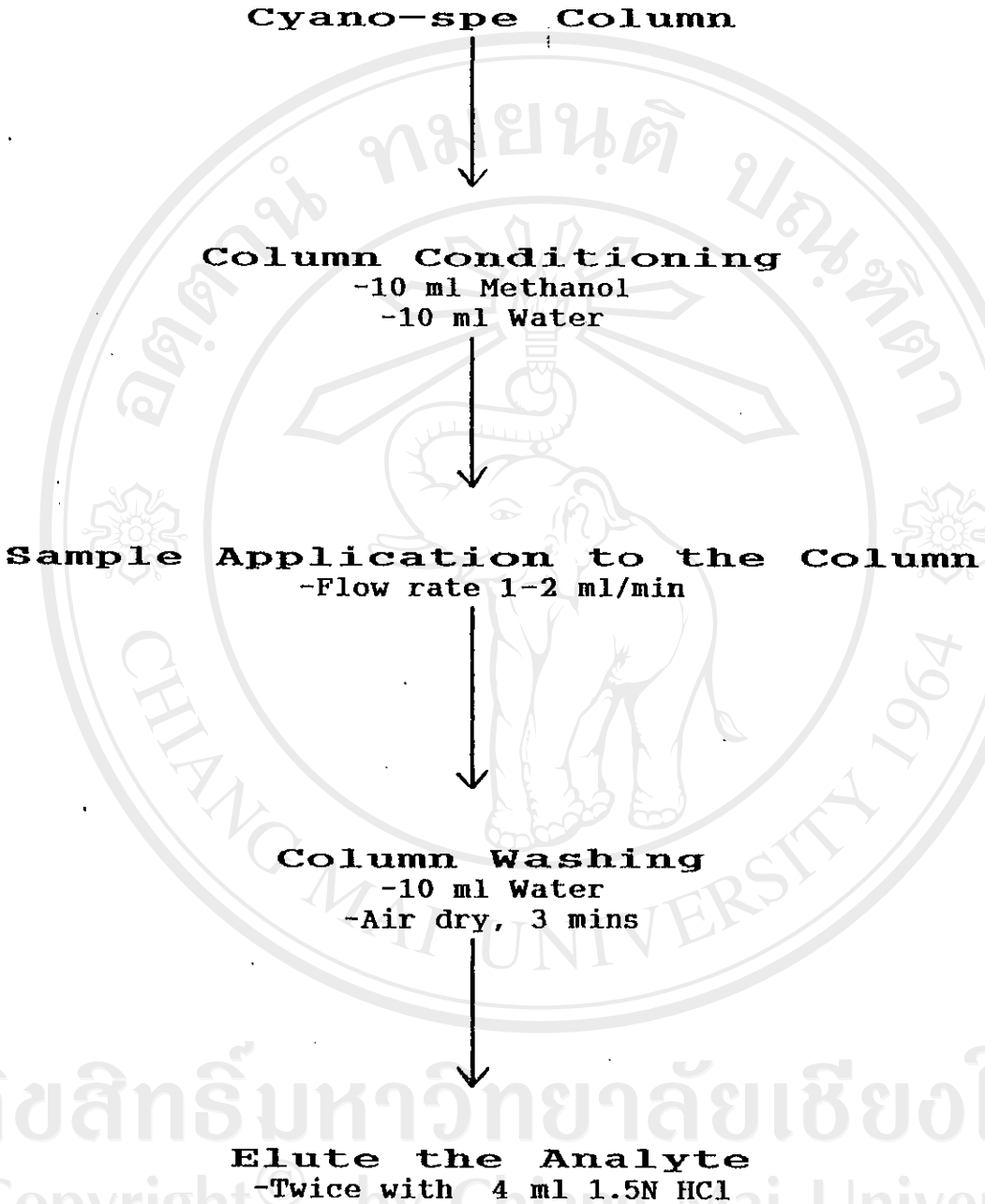

Figure 2.4 The cyano-spe sample preparation flow chart.

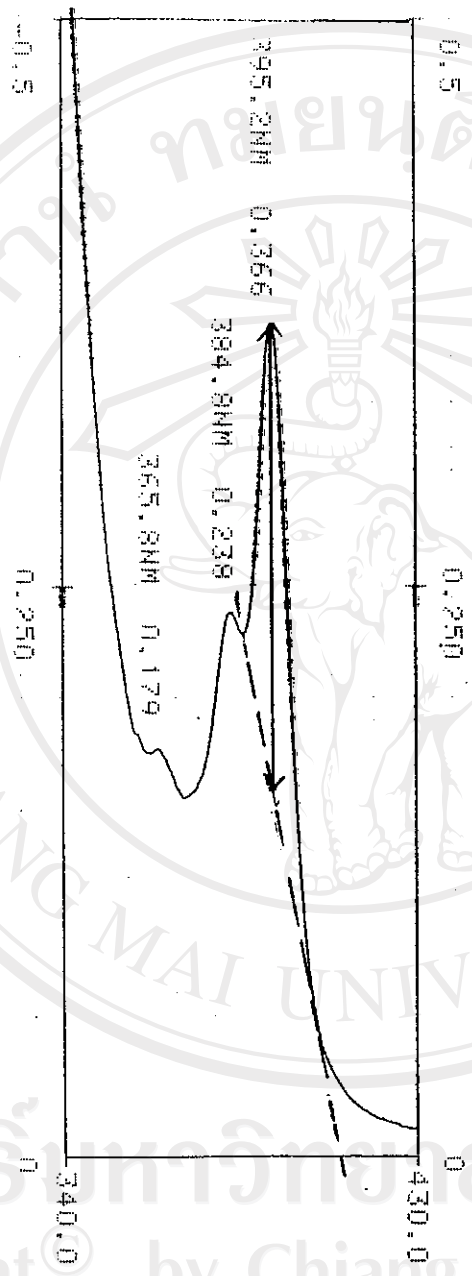

Figure 2.5 Drawing the baseline of the absorption spectrum.

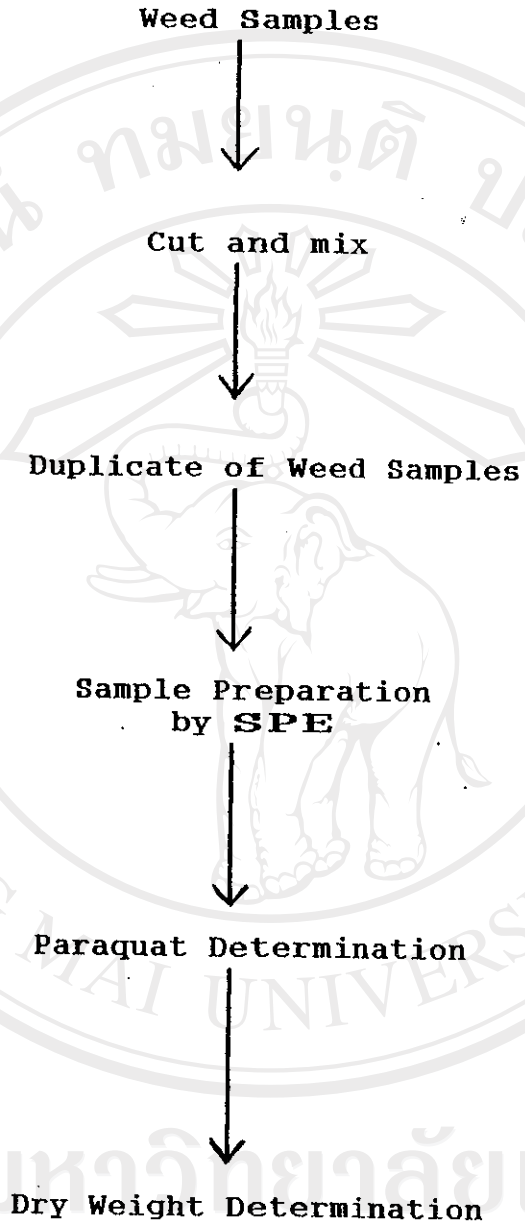

Figure 2.6 Sample analysis flow chart.

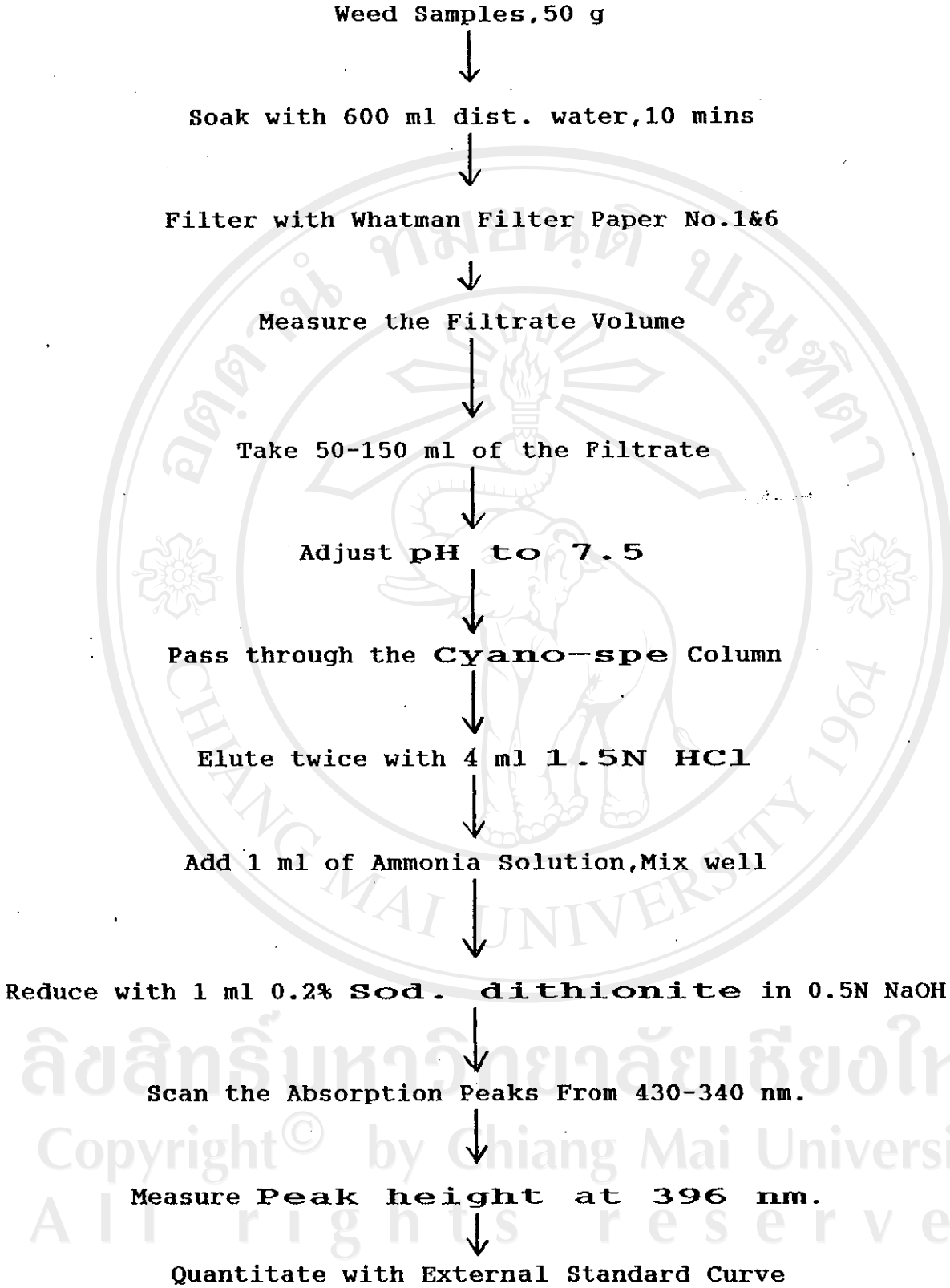

Figure 2.7 Analysis of the deposited paraquat flow chart.

2.11 Determination of the Weed's Dry Weight

Each of the samples was placed in a known weight paper bag and dried in an oven at temperature about 50-60^{0}c until a constant weight was obtained.

The total dry weight of the weed sample before the application in each of the individual trials was then calculated in terms of g/m^2 to represent the average density of the weed at study site.

2.12 Standard Calibration Curve Construction

Three sets of standard calibration curves, i.e, "**low**" range (0.025-0.2 ug/ml), "**medium**" range (0.5-2.0 ug/ml) and "**high**" range (2.0-8.0 ug/ml) were obtained by plotting the mean values (n=3) of the peak heights obtained by the same procedure as that for the sample analysis in Section 2.10.

2.13 Stability of Paraquat in Weed Extracting Water

A 0.4 ml volume of the stock standard solution of paraquat (250 ug/ml) was transferred into a 100 ml volumetric flask and added with the weed extracting water to the mark. The solution was well mixed before being kept in a plastic container and stored at room temperature. The amount of paraquat in the solution was determined by following the sample analysis steps in Section 2.10.

2.14 Determination of the Linear Range for the Standard Paraquat

The average (n=2) absorbance values at 396 nm of the standard paraquat cation, of concentrations from 2.5-40 ug/ml

were plotted against their corresponding concentrations in order to locate the linear range of absorbance.

2.15 Percentage of Recovery

2.15.1 Percent recovery of 2 ug paraquat in weed extracting water

A duplicate of 200 ml volume of weed extracting water which spiked with 20 ul of the working standard solution (100 ug/ml) was concentrated by the SPE prior to the determination of paraquat by following the sample analysis steps in Section 2.10 and then the average percent recovery was calculated accordingly.

2.15.2 Percent recovery of 30 ug paraquat in weed extracting water

This percentage recovery was obtained by the same procedure as that described in Section 2.15.1 except that a 120 ul volume of the stock solution of paraquat (250 ug/ml) was spiked.

2.16 Detection Limit

2.16.1 The lower limit of detection[43]

Series of concentrations of paraquat (0.001-0.025 ug/ml) were prepared in the mixture solution of 1.5M hydrochloric acid and ammonia solution (4:1 v/v). The absorption peak was scanned under the conditions identical to those in the sample analysis (Section 2.10). The lowest concentration of paraquat which produced a reliable absorption peak detected by the instrum was recorded.

2.16.2 The instrumental detection limit (S/N=2)

The average height (n=6) of the blank baseline was multiplied by two and then the value obtained was compared with the corresponding standard calibration curve to determine the amount of paraquat.

2.17 Limit of Determination

The limit of determination of this analysis procedure was calculated under the following analysis conditions: 50 g of weed sample taken from a total 500 g weed samples (their dry weights were 15 and 180 g, respectively); 150 ml of the filtrate taken from the total of 600 ml was passed through the cyano-spe column; 5 ml of eluate and the detection limit (S/N=2) was 0.015 ug/ml.

2.18 Stability of the Reduced Paraquat Ion

A 5 ml volume of 0.5 and 2.0 ug/ml of the working standard paraquat were reduced separately with 1 ml of 0.2% sodium dithionite in 0.5M sodium hydroxide, mixed by gently inverting 3 times and then the absorption was measured at 396 nm immediately and at the interval of time 0, 2.5, 5, 7.5, 10, 15, 20 and 30 minutes after reduction.

2.19 Absorption of the Bakerbond Cyano-spe and the Paraquat Free Weed Extracting Water

A 200 ml volume of distilled water and the paraquat free weed extracting water were cleaned up by the cyano-spe and then analyzed by following Section 2.10 in order to determine whether or not the absorption of these two matrices

interfered the analysis.

2.20 Repeatability of the Absorbance Measurement

Ten replicates of 2.0 ug/ml working standard solution were reduced according to the procedure in Section 2.18 and the absorbance was measured at 396 nm.

2.21 Calculation

2.21.1 Paraquat in the replicate weed sample

The amount of paraquat in each individual analyte was calculated from the following equation.

$$PQ_A \text{ (mg/kg)} = \frac{[E_{1A} + E_{2A}]VT}{W_A x V_A} \quad \text{----(2.1)}$$

$$PQ_A \text{ (g/ha)} = \frac{[E_{1A} + E_{2A}]\ VTxWTx10^{-2}}{V_A\ xW_A x0.75} \quad \text{--(2.2)}$$

where PQ_A = Amount of deposited paraquat on the replicate A, mg/kg or g/ha.

E_{1A} , E_{2A} = Amount of paraquat from the first and second elution, ug.

VT = Total volume of the weed extracting water of replicate A, ml.

V_A = Volume of the weed extracting water of replicate A used, ml.

WT = Total dry weight of weed sample in the corresponding plot, g.

W_A = Dry weight of weed sample replicate A after extraction, g.

2.21.2 Paraquat in the study plot.

The amount of paraquat in each plot was calculated by the following equation.

$$PQ_1 = \frac{[PQ_A + PQ_B]}{2} \qquad \text{-----(2.3)}$$

where PQ_1 = Amount of paraquat in plot no.1, mg/kg or g/ha.

PQ_A , PQ_B = Amount of paraquat in the replicate weed sample of the corresponding plot, mg/kg or g/ha.

2.21.3 Paraquat in each trial

The amount of paraquat in each trial study was calculated by the following equation.

$$PQ_V = \frac{[PQ_1 + PQ_2 + PQ_3]}{3} \qquad \text{----(2.4)}$$

where PQ_V = Amount of paraquat in trial V, mg/kg or g/ha.

PQ_1, PQ_2, PQ_3 = Amount of paraquat in plot no.1, 2 and 3 of the corresponding trial, mg/kg or g/ha, respectively.

2.21.4 Paraquat for each of the two practices

$$PQ_P = \frac{[PQ_V + PQ_R]}{2} \qquad \text{----(2.5)}$$

where PQ_P = Amount of paraquat after application by the

farmer's practice, mg/kg or g/ha.

PQ_V, PQ_R = Average amount of paraquat at two trials , V and L, after application by the farmer's practice, mg/kg or g/ha.