# CHAPTER II

## MATERIALS AND METHODS

### 1. MATERIALS

The following substances were obtained from commercial sources except for Durian rind extract.

active ingredients :

- hydrochlorothiazide (China National Chemicals Imp & Exp Co., China)
- pyridoxine hydrochloride (BASF,Germany)

diluents :

- α-lactose monohydrate (Tablettose®, Meggle Milch Industrial GMBH & Co, Germany)
- dibasic calcium phosphate dihydrate (Emcompress®, Edward Mendell Co. New York, USA)

disintegrants :

- durian rind extract ($D_1$ ,alcohol extraction : $D_2$, acid-alcohol extraction) (Biochemistry Lab., Chulalongkorn University)
- corn starch (Pharmaceutical Sciences, Bangkok, Thailand)
- Starch 1500®, (Colorcon Inc, USA)
- Explotab® (Edward Mendell Co. New York, USA)
- Ac-di-sol® (FMC, Philiadelphia, USA)
- Nymcel® (ZSB 16, Nyma, Netherland)
- Kollidon CL® (BASF, Germany)

lubricant :

- magnesium stearate (Pharmaceutical Sciences, Bangkok, Thailand)

miscellaneous :

- Ethanol, Absolute (E.Merck, Darmstadt, Germany)
- Hydrochloric acid, analytical grade (E.Merck, Darmstadt, Germany)

## 2. EQUIPMENTS

- Single punch tablet machine (Hanseaten Type E1, Germany)
- Strain meter (TML Instruments, Tokyo Sokki Kenkyujo Co., LTD., Japan)
- Strain indicator amplifier (Kyowa, Type DPM-612A, Japan)
- Desk-Top type automatic self-balancing recorder (Nippon Denshi Kagaku Co., Ltd., Model U-631, Japan)
- Malvern particle sizers (Malvern, Series 2600C, UK)
- analytical balance (Sartorius, Germany)
- scanning electron microscope (Jeol, JSM-35CF, Japan)
- centrifuge apparatus (Sigma, Model 302K, Germany)
- moisture determination balance (Ohaus Scale Corp., USA)
- hardness tester (Schleuniger 2E, Germany)
- micrometer (Teclock Corp., 0.01 mm., Japan)
- Roche friabilator (Erweka, Germany)
- disintegration apparatus (Hanson Research, USA)
- dissolution apparatus (Hanson Research, USA)
- spectrophotometer (The Bausch & Lomb, New York, USA)

# 3. METHODS

## 3.1 Preparation of Durian Rind Extract (11)

- $D_1$ (Alcohol Extraction)
- $D_2$ (Acid-Alcohol Extraction)

## 3.2 Physical Properties of Disintegrants

### 3.2.1 Size and shape from scanning electron microscope

Samples of each disintegrant were examined for size and shape under a scanning electron microscope (SEM) at appropriate magnifications and photographs were taken.

### 3.2.2 Size distribution and specific surface area

Samples of each disintegrant were examined for size distribution, cumulative distribution, specific surface area and average diameters of particle by laser diffraction, using Malvern particle sizers Series 2600 C. The data were collected and shown by computer.

### 3.2.3 Bulk and tapped density

Bulk density was performed by pouring 25 g material into a 100 ml graduated cylinder and measuring the volume to the nearest ml. Tapped density was performed by dropping graduated cylinder onto a hard wood surface from a height of 5 cm until the powder attained a constant tapped volume. The results present an average of two determinations per sample.

### 3.2.4 Moisture Determination

Each sample was accurately weighed 1 g on a pan of Ohaus moisture determination balance; dried at dial setting No. 4 ($\simeq$150 °c) until the powder attained a constant weight. The

percentage of moisture loss was calculated based on the following equation:

$$\text{Percentage of Moisture Loss} = \frac{(\text{Wet Sample Mass} - \text{Dry Sample Mass})}{\text{Wet Sample Mass}} \times 100 \quad (7)$$

### 3.2.5 Flow Rate

Each sample was accurately weighed 50 g and filled in funnel which had orifice about 6 mm. Time was recorded since the sample was permitted to flow already. Flow rate was calculated in g/sec. The results present an average of two determinations per sample.

### 3.2.6 Hydration Capacity

A 2 g sample of disintegrant was placed into a 100 ml centrifuge tube tared with stopper. Forty milliliters of water (pH 6-7) was added, and the tube was stoppered and shaken vigorously to suspend the sample thoroughly. The suspension was allowed to stand for 10 min. During this time, it was mixed by inverting three times at the end of 5 and 10 min. The stopper was then removed and the tube was centrifuged for 15 min at 1,000 xg. The centrifuge was allowed to stop without braking. The supernate was carefully decanted and the tube was inverted to allow draining. The tube was then restoppered and the contents were weighed. The hydration capacity was calculated in the following manner:

$$\text{hydration capacity} = \frac{(\text{weight of tube+sediment}) - (\text{weight of tube})}{\text{sample weight (dry basis)}} \quad (8)$$

### 3.2.7 Swelling of particles

Specimens of monodispersed material at least 30 particles were prepared in dry condition and photomicrographs

were taken. Water and Ø.1 N.HCl which have been chosen as swelling media were dropped to the same specimens and photomicrographs were taken again. The swelling index of particles was calculated according to Caramella et. al method(2Ø) as given in Figure 1.

### 3.3 Calibration of the Instrumentation for the Tablettting Machine

The strain gages mounted on the upper punch holder were calibrated under static condition by using hydraulic press over a range of force between 1ØØ up to 1,6ØØ kg.

A linear relationship between strain (um) and applied forces was found with correlation coefficient of Ø.9999 as shown in Table 2 and Figure 2.

### 3.4 Preparation of Tablets

#### 3.4.1 Preparation of tablets without active ingredient

All disintegrants and magnesium stearate were passed through No. 8Ø handle screen, then each excipient was dried in hot air oven at 7Ø °C for 3Ø minutes. A batch of 3ØØ g of various formulations from Table 3 were prepared by mixing with two types of direct compression diluents; α-lactose monohydrate and dibasic calcium phosphate dihydrate, and with eight disintegrants, using a laboratory scale cube mixer at rotation speed of 5Ø rpm. After mixing for 1Ø minutes magnesium stearate was then added, as lubricant, mixing procedure continue for another 5 minutes. Tablets were compressed using an instrumented single punch tablet machine tooled with 8 mm round flat faced punch. The tablet weight of each formulation was 25Ø mg. Each

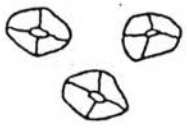
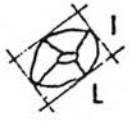
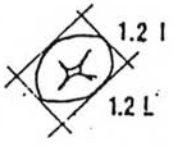
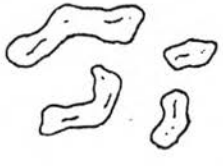
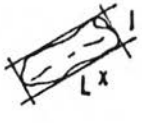
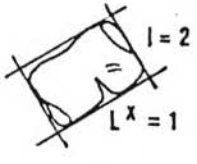
| PARTICLE SHAPE | APPROXIMATED PARTICLE GEOMETRY | INERT MEDIUM no swelling | AQUEOUS MEDIUM | SWELLING INDEX |
|---|---|---|---|---|
| Ovoidal | Prolate ellipsoids | | Symmetrical swelling | |
|  |  |   |   | |
| | $V = \frac{\pi}{6} l^2 L$ | | | |
| | $d_V = \sqrt[3]{l^2 L}$ | $d_V = \sqrt[3]{l^2 L}$ | $d_V = 1.2 \sqrt[3]{l^2 L}$ | $S.I. = \frac{\bar{d}^*_{V\,\text{aqueous medium}}}{\bar{d}^*_{V\,\text{inert medium}}}$ |
| Fiber-like, Rod -like | Cylinders | | Radial swelling only | |
|  |  |   |   | |
| | $V = \frac{\pi}{4} l^2 L$ | | | |
| | $d_V = \sqrt[3]{\frac{3}{2} l^2 L}$ | $d_V = \sqrt[3]{\frac{3}{2} l^2 L}$ | $d_V = 1.58 \sqrt[3]{\frac{3}{2} l^2 L}$ | $S.I. = \frac{\bar{d}^*_{V\,\text{aqueous medium}}}{\bar{d}^*_{V\,\text{inert medium}}}$ |

Figure 1 Evaluation of swelling index of monodispersed materials

Table 2

The Calibration Data Between Strain and Applied Forces Obtained from the Upper Punch.

| FORCE (KG) | STRAIN (μm) |
|---|---|
| 0 | 0 |
| 101 | 20 |
| 200 | 40 |
| 400 | 81.5 |
| 600 | 122.5 |
| 800 | 163 |
| 997.5 | 204.5 |
| 1197.5 | 247 |
| 1400 | 287.5 |
| 1600 | 331.25 |

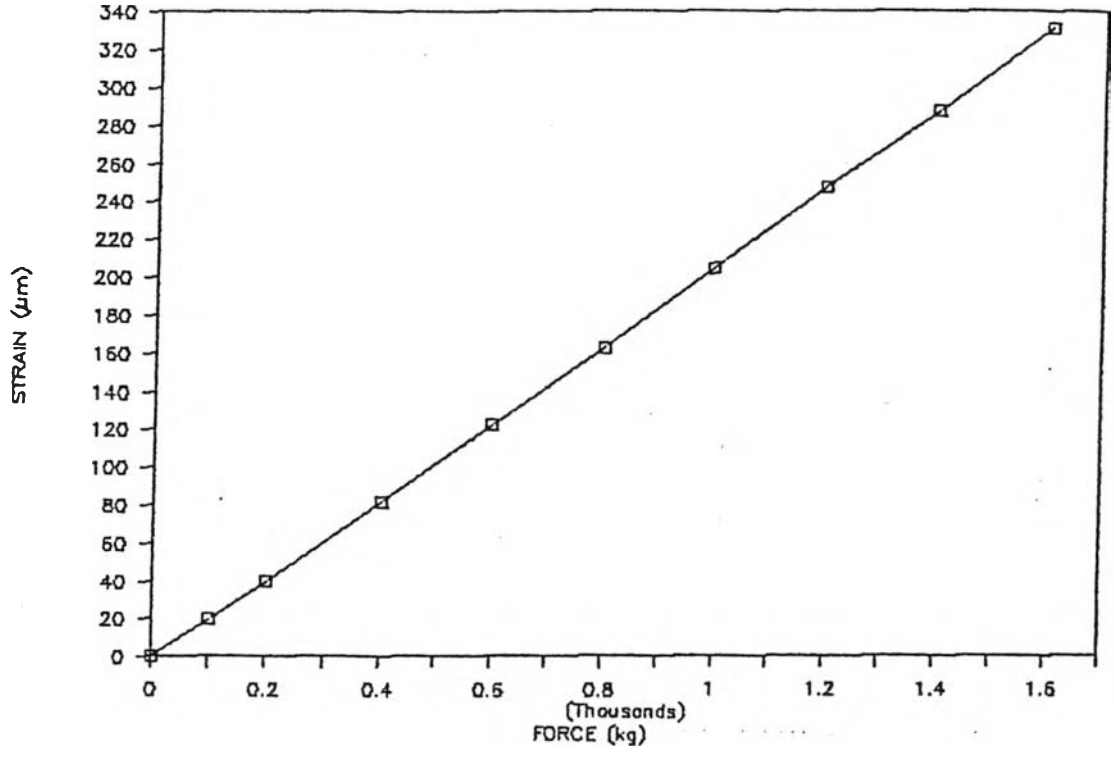



Figure 2 The calibration curve of the upper punch

$y = 0.207x - 1.1311$

Table 3

Tablet Compositions for Evaluation of Disintegrant Efficacy

| | % w/w per tablet | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Series 1 | | | | |
| Emcompress(R) | 98.5 | 98 | 97 | 94 |
| disintegrant* | 0.5 | 1 | 2 | 5 |
| magnesium stearate | 1 | 1 | 1 | 1 |
| Series 2 | | | | |
| Tablettose(R) | 98.5 | 98 | 97 | 94 |
| disintegrant* | 0.5 | 1 | 2 | 5 |
| magnesium stearate | 1 | 1 | 1 | 1 |

Batch size 300 g; Tablet weight 250 mg; Compressional forces : 500, 1000 and 1500 kg

* disintegrant: $D_1$, $D_2$, Ac-di-Sol(R), corn starch, Explotab(R), Kollidon CL(R), Nymcel(R), Starch 1500(R)

batch from each formulation was compressed at three different compressional forces of 500, 1000 and 1500 kg.

3.4.2 Preparation of Hydrochlorothiazide and Pyridoxine hydrochloride Tablets

Durian Rind Extract ($D_1$ and $D_2$) and magnesium stearate were passed through No. 80 handle screen. A batch of 300 g of various formulations from Table 4 were prepared by mixing dibasic calcium phosphate dihydrate, active ingredient and disintegrant using a laboratory scale cube mixer at rotation speed of 50 rpm. After mixing for 10 minutes magnesium stearate was added, as lubricant, mixing procedure continue for another 5 minutes. Tablets were compressed using an instrumented single punch machine tooled with 3/8 inch concave punch. The tablet weight of each formulation was 350 mg. Hardness were controlled within the range of 7 ± 1 kp.

3.5 Tablet Evaluation

3.5.1 Weight variation : Twenty tablets from each batch were weighed individually and determine for average weigh and standard deviation.

3.5.2 Hardness : Hardness of the tablets was determined by using a Schleuniger-2E Hardness Tester and expressed in Kilopound units. The hardness was an average of twenty determinations.

3.5.3 Thickness : Thickness of the tablets was determined by using a micrometer and expressed in mm. The *thickness was an average of twenty determinations.

Table 4

Tablet Compositions with Active Ingredients For Evaluation of Disintegrant Efficacy.

| | % w/w per tablet | | |
|---|---|---|---|
| | A | B | C |
| Series 1 | | | |
| Emcompress(R) | 87 | 86 | 85 |
| hydrochlorothiazide | 12 | 12 | 12 |
| disintegrant* | 0 | 1 | 2 |
| magnesium stearate | 1 | 1 | 1 |
| Series 2 | | | |
| Emcompress(R) | 93 | 92 | 91 |
| Pyridoxine hydrochloride | 6 | 6 | 6 |
| disintegrant* | 0 | 1 | 2 |
| magnesium stearate | 1 | 1 | 1 |

Batch size 300 g; Tablet weight 350 mg; Hardness 7 ± 1 kp.

*disintegrant : $D_1$, $D_2$, corn starch, Explotab(R)

3.5.4 Friability : A sample of tablets consisting of not less than 20 tablets nor less than 6.0 g were weighed. The friabilator was rotated at 25 rpm for 4 minutes. Then sample were reweighed. Friability were calculated as percent weight loss.

3.5.5 Water Uptake : The apparatus consists of a sintered glass filter connected to a horizontal graduated pipette by a rubber bung as showed in Figure 3. The entire assembly was immersed in a water bath, thermostatically controlled at 37 ± 1 °C. A continuous water volume was maintained from the sintered glass filter through the glass bottle to the end of the pipette. A piece of filter paper was placed on top of the sintered glass base. The top of rubber bung was covered with slide to prevent evaporating of penetration liquid. The volume of water uptake was read from the graduated pipette. Then mean of at least five determinations was taken to represent the uptake volume.

3.5.7 Disintegration times : Disintegration time of the tablets was determined in distilled water using Disintegration Apparatus according to the USP XXI method. This value was measured in seconds. The means of at least six determinations of each batch was presented.

3.5.8 Dissolution Times :

Dissolution time of tablets was determined using a Dissolution Test Apparatus according to USP XXI method.

Procedure : 900 ml of 0.1 N hydrochloric acid for hydrochlorothiazide tablets and dilute hydrochloric acid (1 in 100) for pyridoxine HCl tablets were placed in the vessel and permitted to equilibrate to 37° ± 0.5° C. Place a tablet in the basket and immerse the basket into the vessel. The basket were

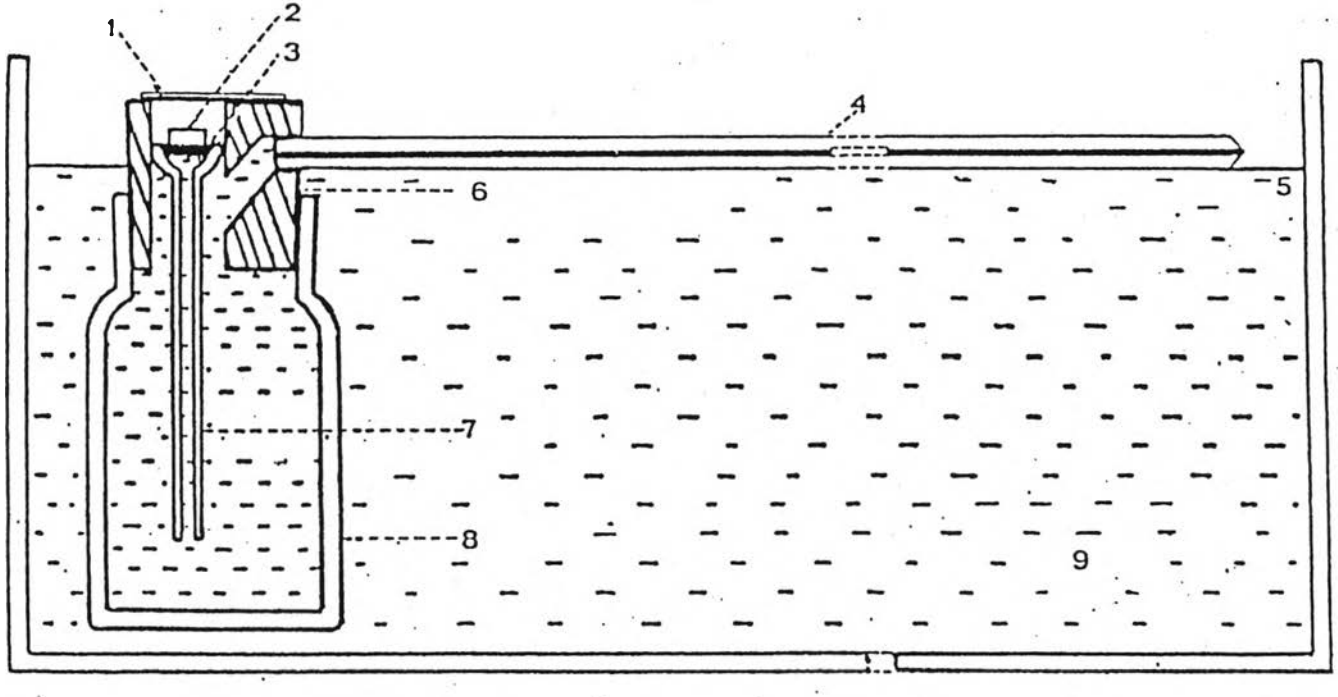



Figure 3. Apparatus for determination of water penetration into tablets. 1. cover slide 2. tablet 3. filter paper 4. pipette 5. water level 6. rubber bung 7. filter stick 8. glass bottle 9. thermostated at 37 °C

rotated at the speed of 150 rpm for hydrochlorothiazide and 100 rpm for pyridoxine HCl.

At predetermined time intervals (5, 10, 15, 20 and 30 min), 5 ml of the sample solution were withdrawn by an automatic sampling instrument. Then the same quantity of dissolution medium were replaced immediately after each sampling. The dissolution experiment was conducted until 30 minutes. The amount of active ingredient in each sample was analyzed spectrophotometrically compared with standard curve (Figures 4a and 5). Determine the absorbance at 272 nm for hydrochlorothiazide and at 290 nm for pyridoxine HCl.

3.5.9 Content Uniformity

Hydrochlorothiazide : A tablet, previously grind to finely powdered was transferred to a 50 ml volumetric flask containing about 30 ml of ethanol, shake for 10 minutes, and dilute to volume. Filter a portion of the mixture, discarding the first 5 ml of the filtrate. Pipette 0.1 ml of filtrate and dilute to 10 ml of volumetric flask. Determine the absorbance at 269 nm using ethanol as the blank. The amount of hydrochlorothiazide was calculated from standard curve of hydrochlorothiazide in ethanol (Figure 4b) which had been performed before.

Pyridoxine HCl : A tablet, previously grind to finely powdered was transferred to a 500 ml volumetric flask containing about 300 ml of water, shake for about 30 minutes, and dilute with water to volume. Dilute a suitable aliquot of the subsequent filtrate quantitatively and stepwise with dilute hydrochloric acid (1 in 100) and dilute quantitatively and stepwise with the same solvent to obtain a standard solution having a known concentration of about 10 ug per ml. Determine the

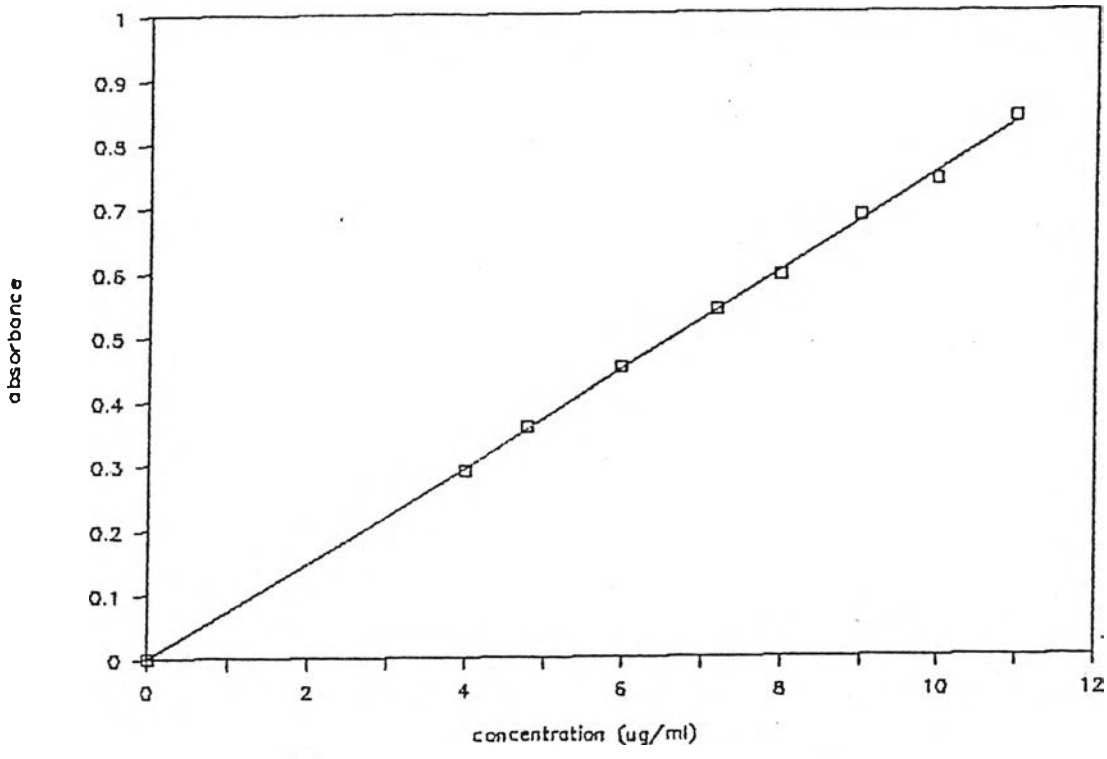



Figure 4a Standard curve for hydrochlorothiazide in 0.1 N hydrochloric acid at 272 nm.

$y = 0.077x - 0.010$

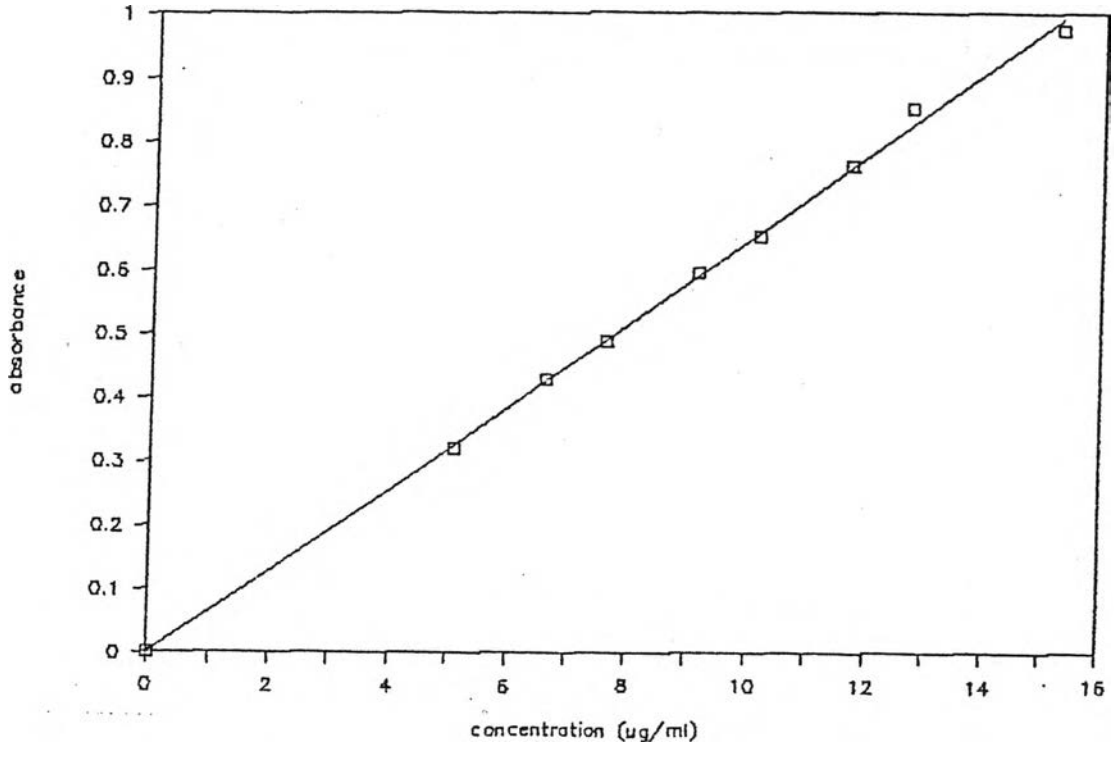



Figure 4b Standard curve for hydrochlorothiazide in ethanol at 269 nm.

$y = 0.066x - 0.0091$

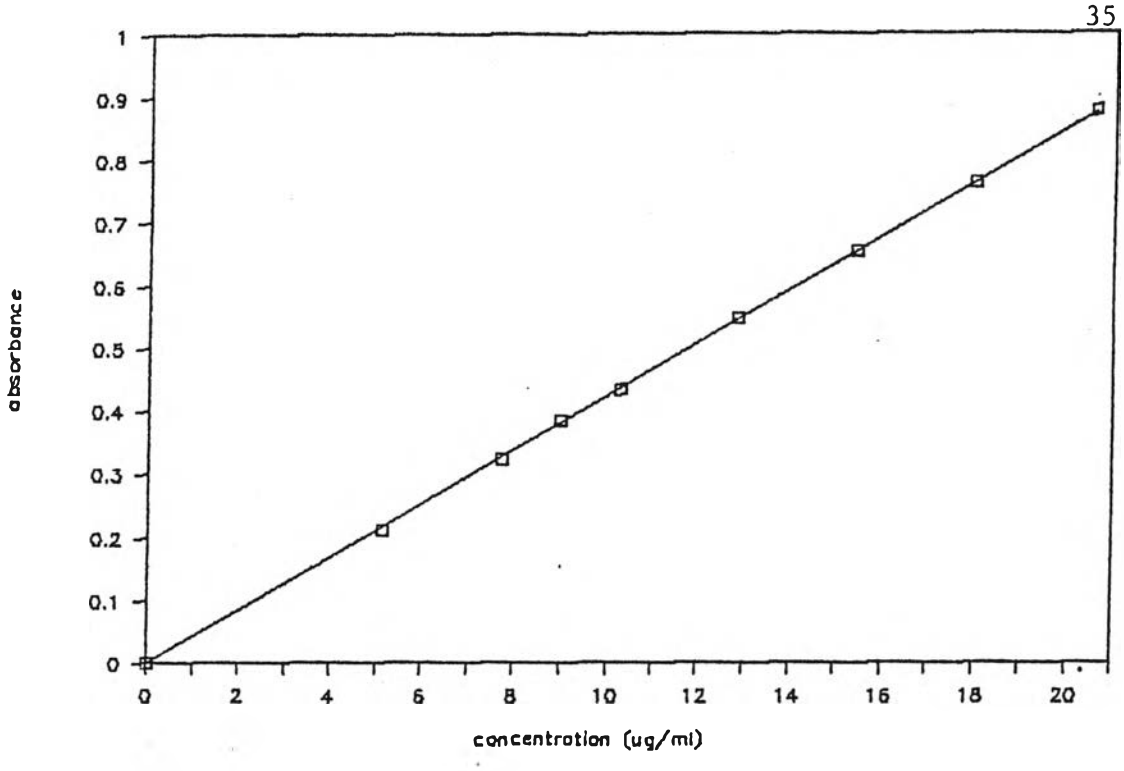



Figure 5 Standard curve for pyridoxine hydrochloride in dilute hydrochloric acid (1 in 100) at 290 nm.

$y = 0.043x - 0.0066$

absorbance at 290 nm using dilute hydrochloric acid (1 in 100) as the blank. The amount of pyridoxine hydrochloride was calculated from standard curve of pyridoxine hydrochloride in dilute hydrochloric acid (1 in 100) which had been performed before (Figure 5).