CHAPTER 2

MATERIALS AND METHODS

1. Materials

The following materials were obtained from commercial sources

1.1 **Model Drug**

Diazepam (Batch No. 31178, China)

1.2 **Additives**

1.2.1 Diluent

Lactose USP XX (Batch No. 16265, DMV vighel Halland)

1.2.2 Binder

Polyvinylpyrrolidone (PVP) (Lot No. 303861, GAF (Singapore) Pte.,Ltd., Singapore.)

1.2.3 Lubricant

Magnesium stearate (Lot No. 9851085, LEK Lynbiyama Yugoslavia)

1.2.4 Glidant

Talcum (Lot No. 194677, I.L Shine Industrial Co.,Ltd.)

2. Equipment

The following equipment were used

2.1 US standard seives no. 20, 40, 60, 80, 100 (Endecotts Ltd., London, England)

2.2 Analytical balance (Sartorius type 2442, Switzerland)

2.3 Balance (Ohous, Flarham Park.N.J., U.S.A.)

2.4 Hot air oven (Lytren Own, Switzerland)

2.5 Manesty disintegration test unit (Manesty Machine Ltd., Liverpual,24 England)

2.6 Dissolution tester (Hanson Research Corp. model 500.230 with dissolution drive control , U.S.A.)

2.7 Spectrophotometer (Spectronic-2000, Baush & lomb, U.S.A.)

2.8 Automatic voltage stabilizer (Quasar model AVS-4002B, U.S.A.)

2.9 Shaker (Type BMK4 1400 u/min, Josef Dechelman Western Germany)

2.10 UniGlatt (Wurster- System, Germany)

2.11 Moisture determinatiom balance (Ohaus Scale corp., U.S.A.)

2.12 Oscillating granulator (KSL,Bangkok, Thailand)

2.13 Single punch (VE, Bangkok, Thailand)

2.14 Hardess tester (Schleuniger-2E Model 2E/205, U.S.A.)

3. Preparation of Granules

Diazepam granules were prepared by using ingredients listed in below formula. A batch of 1,000 tablets was prepared for each method. And each method was repleted 5 times.

The Formulation of Diazepam Tablets

Ingredient	mg/tab
Diazepam	5
Lactose	200
PVP*	6
Talcum	3%
Magnesium stearate	1%

* As 10% in aqueous solution

3.1 Manual Method

The drug and diluent employed in each formulation were individually passed through a 30 mesh sieve to break agglomerate. Diazepam powder was placed in the motar and ground for 3 minutes. Lactose powder was added and mixed thoroughly for 3 minutes. PVP, 10% in water was added to the powder drug. The mixture was mixed thoroughly into damp mass for 10 minutes.

The damp mass was then passed through a 12, 16, 20, 25 or 30 mesh screen. The granules of each size were placed on a tray and dried in a hot air oven for 12 hours

at 52 C. The dried granules were again passed through the same 12, 16, 20, 25 or 80 mesh screen. The granules, the 3% of talcum and 1% of magnesium stearate were then mixed together for 3 minutes by bottle method.

3.2 Oscillating Granulation Method

The diazepam granules were prepared as in the manual granulation method, except that the damp mass was passed through an oscillating granulator using 12, 16, 20, 25 or 30 mesh screen both before and after drying the granules.

3.3 Fluid Bed Spray Drying Method

The granules were prepared by using a fluid bed spray dryer. The amount of drug and diluent were employed as in the previous method. The amount binder used was 6, 9, 12 or 15 gm. They were prepared as 12% w/v PVP in aqueous solution for each batch. The granulation parameters were kept constant for each amount of binder for 5 batches as followed:

Preheated time:	10 minutes
Inlet air temperature:	52 C
Air pressure to the nozzle:	2 bars
Rate of spray binder:	2.3 gm/min
Inlet air flat:	45

The spraying times were approximately 25, 30, 40 and 54 minutes sequency.

4. Preparation of Tablets

The 3% of talcum and 1% of magnesium stearate were mixed with the granule for 3 minutes in a bottle. Then the prepared granules were compressed into tablets on a Stoke's punch tablet machine, using slightly concave, 3/8 inch punches. The weight of tablet was adjusted to 216.3 mg.. The hardness of tablet was controlled to about 3-6 kilopounds.

5. Evaluation of Granule

5.1 Bulk Volume

The granule of 75 gm. was weighed and poured into 250 ml. cylinder volume . The height of the granule was measured.

5.2 Percentage of Fine

The percentage of fine was calculated as the granule which passed the sieve no. 80 in 5 miniutes.

5.3 Mean Size and Size Distribution

A 75 gm. granule was weighed and placed in a shaker containing a set of sieves no. 20, 40, 60, 80 and 100 mesh and a pan underneath the shaker and operated for 5 minutes. The granules which were left on each seive and on the pan were weighed. The mean size of granules on each sieve and tray and the size distribution was calculated.

5.4 Flow Rate

A 75 gm.of granule was placed into a funnel which had a diameter at the mouth of 11 cm.and had a diameter at the tip of 0.65 cm.. The funnel was stood on the holder of 7.5 cm. stand height. The tip of the funnel was closed. Then the granule was released to flat container which had a diameter 9.5 cm. under the funnel, then the time consumed was calculated.

5.5 Repose Angle

The angle at the base of the granule was derived by a fixed-bed cone method as described in chapter 1.

6. Evaluation of Tablets

6.1 Weight Variation

Individual weight of 20 tablets was determined on an analytical balance. The average weight, standard deviation and coefficient of variation were calculated.

6.2 Hardness of Tablet

Twenty tablets were individually subjected to a hardess tester. The mean, standard deviation and coefficient of variation were calculated.

6.3 Disintegration Time

Disintegration time of tablets was determined

by the U.S.P. using Manesty tablet disintegration test unit. Six tablets were used at a time for each test. Distilled water at 37 C was use as the medium.

6.4 Tablet Friability

Friability value was determined by subjecting 20 tablets of known weight to a controlled series of falling shocks in a friabilator, the drum was made to rotate for 4 minutes at 25 rpm. The percentage lossed due to abration was then calculated.

6.5 Content Uniformity

The content uniformity was conducted by placing a tablet in a motar and ground with pestle. A 5 ml. of absolute ethanol was added to the fine powder. The mixture was stirred thoroughly and poured to the 100 ml. volumetric flask. The mortar and pestle were rinsed thoroughly with distilled water to the solution in the flask. The volume was adjusted to 100 ml. with distilled water. The mixture was shaked well for 10 minutes. Ten ml. sample was pipetted and filtered by a 0.45 um filter before measuring the absorbance at the wavelenghth of 312 nm.(49) with ultraviolet spectrophotometer. The amount of diazepam was calculated from the standard absorbance-concentration curve.

The calibration concentration absorbance curve for content uniformity of diazepam was constructed by preparing the standard at the concentration 20, 40, 50,

60, 80 and 100 ug/ml. in 5% absolute ethanol in water. The absorbance was measured at 312 nm. by UV spectrophotometer which equipped with automatic voltage stabilizer. The data were listed in Table 2. The calibration concentration-absorbance curve of diazepam in 5% absolute ethanol in distilled water were shown in Figure 3.

6.6 Dissolution

The dissolution was conducted by using USP dissolution type I method. A 900 ml. of 1:100 HCl in aqueous solution was used as dissolution medium which was maintained at 37 C. The basket containing one tablet was rotated at the speed of 100 rpm. Ten ml. of sample was pipetted after 30 minutes and then filtered through a 0.45 um filter. The absorbances of samples were determined by UV spectrophotometer. The amount of dissolved drug was calculated from the standard curve.

The calibration concentration - absorbance curve for dissolution of diazepam was constructed by preparing the standard solution at the concentrations of 2, 4, 5, 6, 8 and 10 ug/ml in 0.1 N HCl. The absorbance was measured at 242 nm by UV spectrophotometer which equipped with automatic voltage stabilizer(50). The data were listed in Table 3. The calibration concentration-absorbance curve of diazepam in 0.1 N HCl was shown in Figure 4.

Table 1 The absorbance of standard solution of diazepam in 5% absolute ethanol in water at wavelength of 312 nm.

Concentration (ug/ml.)	Absorbance
20	0.175
40	0.335
50	0.416
60	0.498
80	0.666
100	0.827

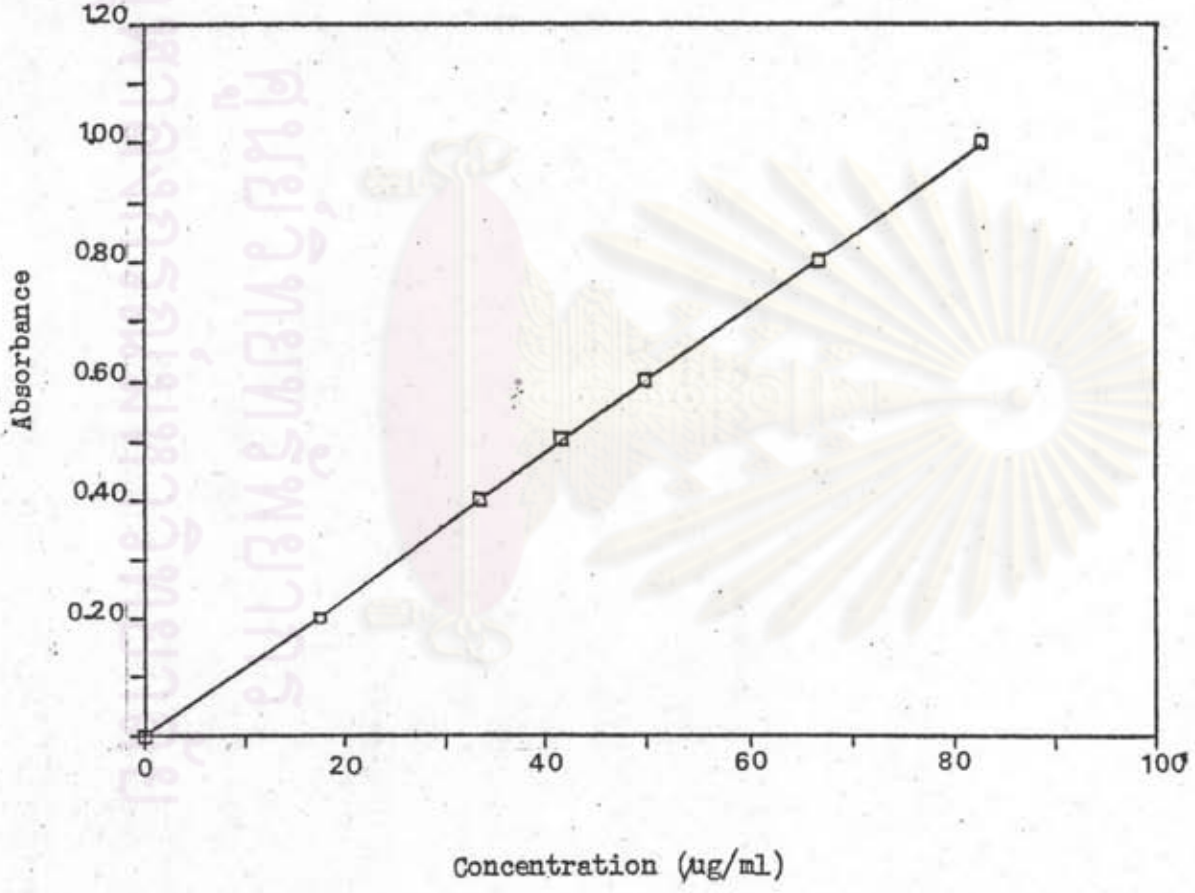

Figure 3 Standard curve of diazepam in 5% ethanol in aqueous solution at the warelength of 312 UM

Table 2 The absorbance of standard solution of diazepam in 0.1 N HCl at the wavelength of 242 nm.

Concentration (μg/ml.)	Absorbance
2	0.222
4	0.470
5	0.537
6	0.643
8	0.850
10	1.058

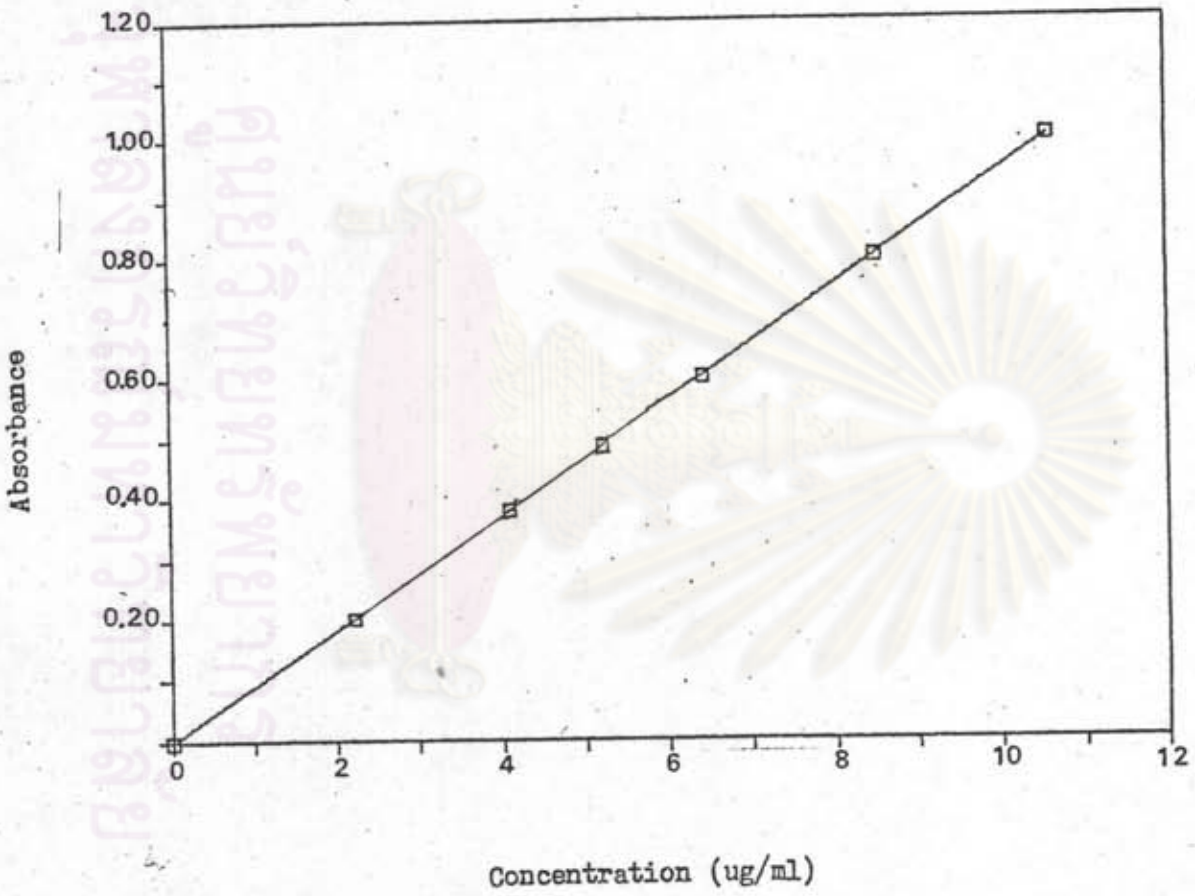

Figure 4 Standard curve of diazepam in 0.1 N HCL at wavelength of 242 UM