Chapter II

Experimental

1. Materials

The following substances were obtained from commercial sources

- Paracetamol (Srichand-United dispensary, Bangkok, Thailand)
- Lactose (The Lactose Company of New Zealand, Hawera, New Zealand)
- Corn starch (Pharmaceutical Sciences, Bangkok, Thailand)
- Sodium starch glycolate (Explotab$^{(R)}$, Edward Mendell, New York, USA.)
- Microcrystalline cellulose (Avicel$^{(R)}$ PH 101, Asahi Chemical Industry, Japan)
- Cross-linked sodium carboxymethyl cellulose (Ac-Di-Sol$^{(R)}$, FMC, Philadelphia, USA)
- Chitin(J) (Tokyo Kasei, TCI, Japan)
- Chitin(U) (Unicord, Bangkok, Thailand)
- Chitosan(J) (Tokyo Kasei, TCI, Japan)
- Chitosan(U) (Unicord, Bangkok, Thailand)
- Magnesium stearate (Pharmaceutical Sciences, Bangkok, Thailand)
- Potassium phosphate, monobasic (E.Merck, Darmstadt, Germany)
- Sodium hydroxide (Riedel-de Haen AG., Germany)
- Methanol, analytical grade (BDH Laboratory Supplies, England)
- Hydrochloric acid (E. Merck, Darmstadt, Germany)

2. Equipment

- Single punch tabletting machine (Yiuhang Engineering, Bangkok, Thailand)
- Strain meter (TML Instruments, Tokyo Sokki Kenkyuju, Japan)
- Amplifier (TML Instruments, Tokyo Sokki Kenkyuju, Japan)
- Recorder (S.C. Siam Engineering, Bangkok, Thailand)
- Analytical balance (Sartorius, Germany)
- Moisture determination balance (Ohaus, USA)
- Hardness tester (Schleuniger, Germany)
- Friabilator (Erweka, Western Germany)
- Disintegration tester (Hanson-Research, USA)
- Dissolution tester model SR_2 (Hanson-Research, USA)
- Unicam SP 1800 Ultraviolet Spectrophotometer (Pye Unicam, Cambridge, England)
- Carver press model C (Perkin Elmer, USA)
- Scanning electron microscope (Jeol,JSM-T 220A, Japan)
- Mechanical sieve shaker (Josef Dèckelmann, Aschaffenburg, Western Germany)
- HPLC pump CM 4000 (Milton Roy, Florida, USA)
- HPLC detector SM 4000 (Milton Roy, Florida, USA)
- Computing integrator CI 4100 (Milton Roy, Florida, USA)
- Zorbax ODS column (Phenomenex, USA)

3. Methods

Some fundamental properties were used to determine

the disintegrating action of the disintegrants. The methods below were employed to assess the physical properties of the materials.

3.1 Evaluation of physical properties of pure disintegrant powders

3.1.1 Particle size and shape

Particle size and shape of the different disintegrants were determined by scanning electron microscope (SEM) at the required magnifications and SEM photomicrographs were taken.

3.1.2 Size distribution

Particle size distribution of each disintegrant was examined by sieve analysis, using a nest of sieve and an electromagnetic sieving machine. US standard sieve No. 40,60,80,100,140,170, and 325 were used. For particle size analysis, a 10 grams sample was placed in the top sieve and screened for 35 minutes on a mechanical sieve shaker. Weight size was the product of the arithmatic mean size of the openings and the percentage retained on the smaller sieve.

3.1.3 Moisture determination

Ten grams of sample were accurately weighed and evenly distributed on a pan of Ohaus moisture determination balance. The temperature was maintained between 105°-110°C (dial setting No.4) until the weight of sample was constant. The percentage of weight or moisture loss on drying was read directly

from the balance. The results were the mean of three determinations.

3.1.4 Swelling of particles

For each sample, five grams were weighed in a 100- ml graduated cylinder. The initial volume (bulk volume) was noted before shaking with 80 millilitres of deionized water and diluted hydrochloric acid (1:100) until all particles were well dispersed. Subsequently, the dispersion was adjusted to volume and was allowed to stand overnight. The sedimentation volume was read and compared. The swelling capacity was the ratio of the sedimentation volume to the bulk volume. The results were the mean of three determinations.

3.2 Evaluation of physical properties of pure disintegrant tablets

3.2.1 Water uptake

The rate and amount of water uptake were determined on a pure disintegrant tablet of 11 mm diameter, 500 mg weight and compressed on flat-face punches at 1500 pounds (Carver hydraulic press).

The determination was carried out using the apparatus shown in Figure 12. It consisted of a sintered glass filter connected with a tubing to a horizontally positioned graduated capillary tube. The entire assembly was immersed in a water bath thermostatically controlled at 37$\pm$ 1°C. To prevent water from entering the

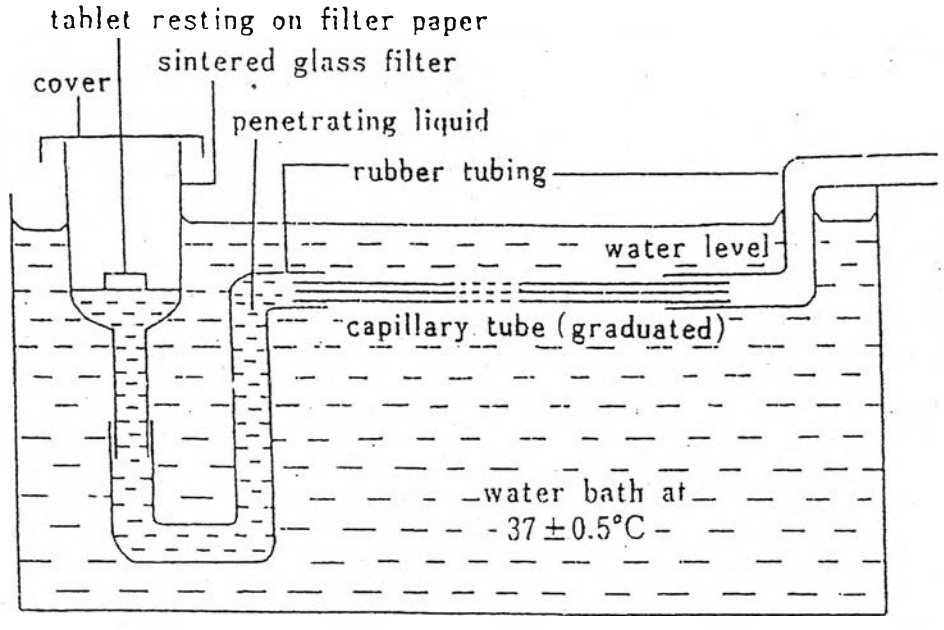


Figure 12 Apparatus for measuring penetration of liquid into tablets

capillary tube, the open end of the capillary was attached to the tubing which was led out of the water-bath. The base of the sintered glass filter was lined with a piece of filter paper of exact size. To prevent loss of water due to evaporation, the glass filter was covered during the entire course of measurement. The change in the length of liquid column in the capillary tube with time was measured. Five replicates of each pure disintegrant tablet were taken.

3.2.2 Moisture sorption

The moisture sorption of tablets compressed on 500 mg of pure sample at 1500 pounds with 11-mm flat face punch (Carver hydraulic press) were determined. About 50 tablets of each sample were accurately weighed and placed in a 60 mm tared glass dish. The sample was placed in a dessicator containing saturated sodium chloride in its reservoir (75% relative humidity). The dessicator was stored at room temperature($26\pm1^{\circ}C$). At various time intervals (2,4,8,24 and 48 hours), the weight gained by the exposed samples were recorded and the amount of moisture sorption was calculated from the weight difference.

3.3 The instrumentation of a tablet machine

The frame of single punch machine was bounded with strain gauge, which used for dynamic force measurements. Two strain gauges, one can active gauge

and the other a temperature compensating gauge, for measuring the applied force from upper punch were mounted to the modified upper punch holder as shown in Figure 13. The active gauge and compensating resistance gauge were formed two arms of a Wheatstone Bridge which connected to one channel of a strain indicator amplifier as shown in Figure 14. When stress was applied, the resulting strain in the upper punch was shown by strain gauges consequently causing a change of resistance of the gauges and an unbalanced in the bridge. The unbalanced potential which was directly proportional to the force applied on upper punch was then amplified by strain indicator amplifier and was recorded on an oscilloscope. The responses were read directly as unit of signal deflection by recorder.

The strain gauges mounted on the upper punch holder were calibrated under static condition by using hydraulic press over a range of force between 300 up to 3,000 pounds. A relationship between strain (microstrain) and applied forces(pounds)was shown in Table 3 and Figure 15.

3.4 Preparation of paracetamol tablet

The compositions of paracetamol tablet formulations were presented in Table 4. All materials were first passed through a No. 40 mesh screen except

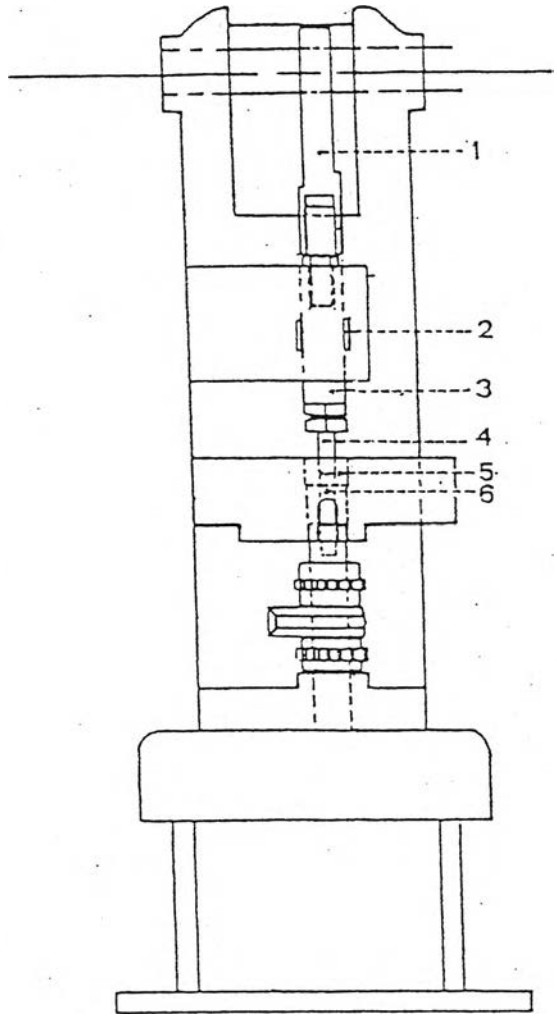


Figure 13 Schematic drawing of the instrumental tablet machine

1. eccentric sheave
2. strain guage
3. upper plunger
4. upper punch
5. die
6. lower punch

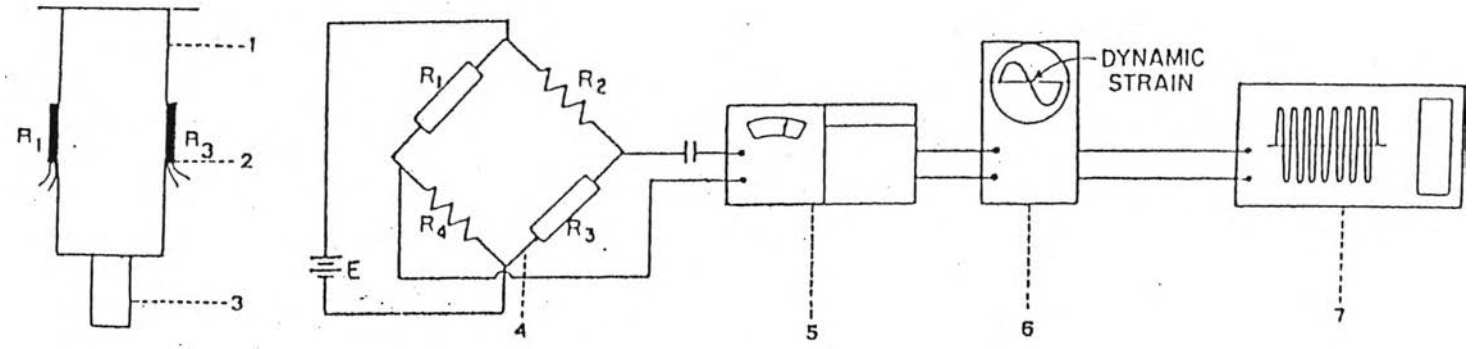


Figure 14 Function block diagram of press and associated measuring system

1. upper plunger
2. strain guage
3. upper punch
4. wheatstone bridge
5. dynamic strain indicator
6. oscilloscope
7. recorder

Table 3 The calibration data between strain and applied forces obtained from the upper punch

Force (pound)	Strain (microctrain)
300	26.58
600	46.82
900	68.25
1200	87.29
1500	109.52
1800	128.57
2100	150.39
2400	169.83
2700	192.45
3000	211.90

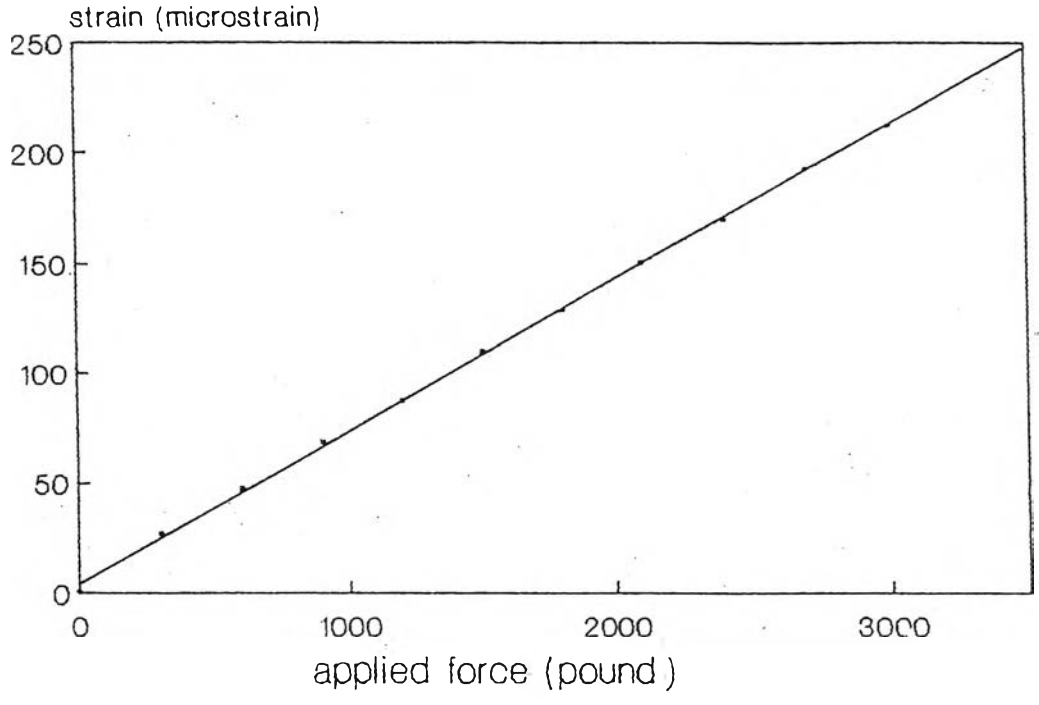


Figure 15 The calibration curve between strain and applied forces obtained from the upper punch

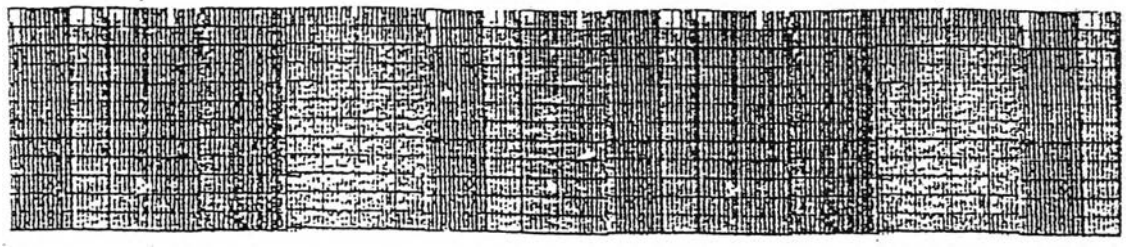
$$Y = 0.066X + 0.003$$

Table 4 The composition of paracetamol tablet formulation

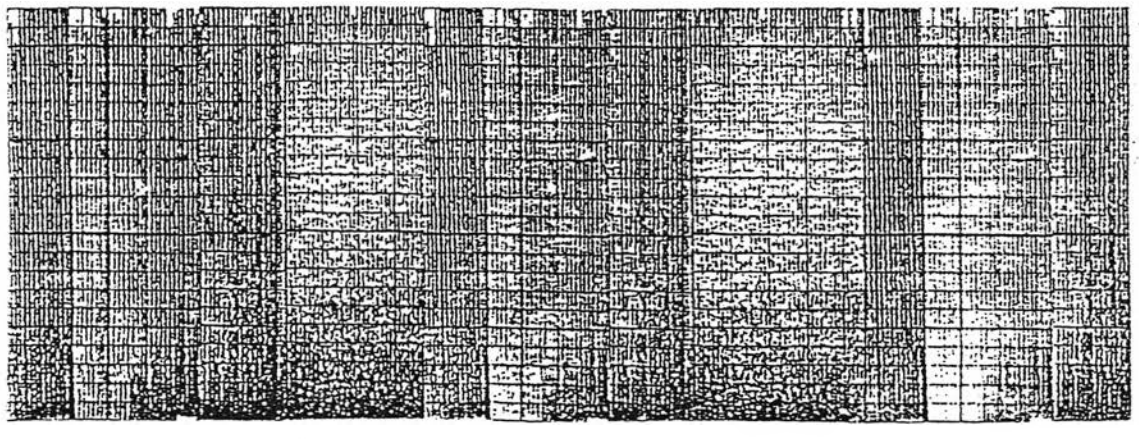
Ingredient	Amount (mg/tablet)
Paracetamol	500
Lactose	5
PVP K30	15
Disintegrant	*
Magnesium stearate	5

* Corn starch	5 %	of drug
Sodium starch glycolate	5 %	"
Microcrystalline cellulose	5,10 %	"
Croscarmellose sodium	2,5 %	"
Chitin (J)	1.5,3,5,7 %	"
Chitin (U)	1.5,3,5,7 %	"
Chitosan (J)	1.5,3,5,7 %	"
Chitosan (U)	1.5,3,5,7 %	"

magnesium stearate which was passed through a No.80 mesh screen. All disintegrants were dried at 50°C for an hour in a hot air oven before granulation. A batch size of about 550 gram of each formulation was prepared. The required amounts of drug, tablet diluent, disintegrant and lubricant were accurately weighed. Drug (paracetamol), diluent (lactose), and intragranular disintegrant (50% of total amount of disintegrant in formulation) were transfered to a Kenwood mixer, and mixed for 5 minutes. The require amount of tablet binder (PVP K30) was accurately weighed and dissolved in a suitable quantity of distilled water. Granulating fluid was added while mixing, and mixing procedure was continued for 5 minutes to produce a mass of proper consistency. The wet mass was passed through a No.12 mesh screen by using an oscillating granulator, and then dried in the hot air oven at 60°C for 3 hours to a moisture level of about 1% w/w. The dried granules were sized through a No.16 mesh screen. The granules were mixed with extragranular disintegrant and magnesium stearate, as lubricant, in a laboratory scale cube mixer at a rotation speed of 50 r.p.m., mixing procedure was continued for 5 minutes. The tablets were compressed on the Stoke's single punch tabletting machine, using 1/2 inch flat faced punch, with compression forces of 600 and 900 pounds. The tracings of the compressional forces were shown in Figure 16. These tablets were used to investigate the physical properties as a function of compression forces before and after exposure to humidity.



(a)



(b)

Figure 16 Tracing of compressional force for all tablet formulations

a) 600 pounds b) 900 pounds

3.5 Evaluation of physical properties of paracetamol tablet

3.5.1 Before exposure to humidity cycling

3.5.1.1 Weight variation

Weight of 20 tablets was individually determined by using an analytical balance and the average weight,standard deviation and percent coefficient of variation were calculated.

3.5.1.2 Hardness

Hardness of the tablets was determined by using a Schleuniger-2E hardness tester, expressed in kilopound unit. The results were the mean of 10 determinations.

3.5.1.3 Friability

A Roche friabilator was employed to determine the tablet friability. The apparatus was rotated at 25 r.p.m.. Twenty previously weighed tablets were placed in the apparatus, and the apparatus was then rotated for 4 minutes to subject the tablets to 100 drops. The tablets were then weighed and the weight loss was calculated in terms of percent friability.

3.5.1.4 Disintegration time

The disintegration time was determined using the USP XXII apparatus. One tablet was placed in each of the six tubes of the basket, a disk was added to each tube, and the apparatus was operated, using deionized water maintained at $37\pm2^{\circ}$ C as the immersion fluid. The value was measured in seconds. The results were the mean of six determinations.

3.5.1.5 Percent drug dissolved

The apparatus utilized was described in USP XXII utilizing a 1000 - ml vessels as the immersion fluid container. A volume of 900 ml of pH 5.8 phosphate buffer solution was used as the dissolution medium. The dissolution medium was equilibrated to 37±1°C. Paddles were used for agitation and a stirring speed of 50 r.p.m. was maintained.

One tablet was placed in each vessel, and the apparatus was immediately operated at the rate specified. Dissolution of the drug from the tablet was conducted through a thirty minute interval. The analysis following dissolution involved the withdrawal of 5 millilitre aliquots at five minute intervals. Then 5 ml of the dissolution medium which had been equilibrated at 37°C was added to the vessel to replace the withdrawal volume. The aliquot was diluted with the dissolution medium to a suitable volume. The absorption of the diluted solution was measured on a spectrophotometer over the Ultra-Violet spectrum at the maximum absorbance, 243 nm , using pH 5.8 phosphate buffer solution as a blank. The percent drug dissolved at a particular time interval was calculated from a standard curve of paracetamol in pH 5.8 phosphate buffer solution at 243 nm , shown in Table 5 and Figure 17.

Table 5 Standard of paracetamol in pH 5.8 phosphate buffer solution at 243 nm

Concentration (μg/ml)	A_{243}
2.00	0.135
3.00	0.200
4.00	0.269
5.00	0.336
6.00	0.401
8.00	0.535
10.00	0.669
12.00	0.793

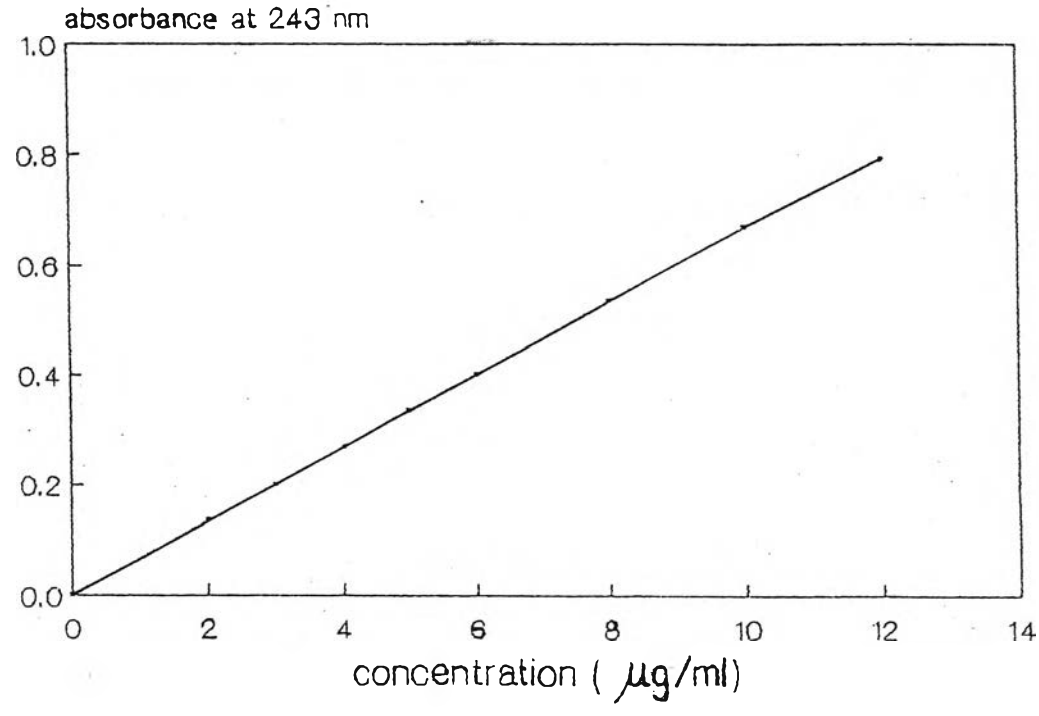


Figure 17 Standard curve for paracetamol in pH 5.8 phosphate buffer at 243 nm

$$Y = 0.066X + 0.003$$

3.5.1.6 Percent labeled amount

In this study, the analysis of percent labeled amount of paracetamol in tablet was determined by HPLC method.

Internal standard solution : An accurately weighed quantity of diclofenac sodium was dissolved in the solvent mixture (40% methanol, 60% water and 0.1% phosphoric acid) and diluted quantitatively to make concentration about 4 mg/ml.

Standard preparation : A 100 mg of paracetamol was accurately weighed into a 50-ml volumetric flask and dissolved with the solvent mixture then diluted quantitatively to volume and mixed. The solution of 0.25, 0.50, 0.75, and 1.00 ml was transfered to a separated 25-ml volumetric flask, then adjusted to volume with the solvent mixture, and thouroughly mixed.

Sample preparation: Not less than 20 tablets of paracetamol were weighed and finely powdered, then average weight per tablet was calculated. A portion of the powder was accurately weighed, equivalent to about 500 mg of paracetamol and transfered with the aid of the solvent mixture to a 50-ml volumetric flask. The mixture was swirled mechanical mean for 30 minutes, diluted with the solvent mixture, mixed and then filtered the solution. The filtrate of 0.25 ml was transfered to a 25-ml volumetric flask, then adjusted to volume with the solvent mixture, and thouroughly mixed.

Chromatographic condition : The mobile phase consisted of 40% methanol, 60% distilled water and 0.1% phosphoric acid was also filtered through a 0.45 μm membrane filter. A flow rate of 1.2 ml/min and the absorbance at 254 nm were established.

Procedure : A 0.5 ml of each standard preparation and 0.2 ml of sample preparation was pipetted to a separated 10-ml volumetric flask. A 1.0 ml of internal standard solution was added to every volumetric flasks, then adjusted to volume with the solvent mixture, and the solution was mixed. Triplicate, 20 μl injections were made from each sample solution and the results were calculated by reference to a standard preparation. The response of the standard preparation was determined by making duplicate.

3.5.2 Exposure to humidity cycling

About 100 tablets containing each disintegrant were placed in a glass dish and exposed to 45°C and 75% relative humidity. At time intervals, 5 days, the tablets were removed for determination of their physical properties as in 3.5.1.