CHAPTER II

MATERIALS AND METHODS

Materials

A. Test products

1. Elixir : The original brand of paracetamol elixir was selected. The concentration of paracetamol elixir was 120 mg/5ml.

2. Suspension : Four commercially available paracetamol suspensions were evaluated. One was the original brand which was assigned as the reference standard against the other three local manufactured brands. The concentration of paracetamol in the suspensions was also 120 mg/5ml.

The letters E, S1, S2, S3 and S4 were given to represent the brand names of each product. Other information of them was listed in Appendix C.

B. Reagents

1. Standard paracetamol powder 99.9 % (Hoechst Pharmaceutical Ind., Ltd.,Bangkok) Batch No. 383 L 648

2. Concentrated Hydrochloric acid 37%, GR grade (Merck) Lot. 445 k5004417.

3. Sodium nitrite, AR grade (May & Baker) Lot.39006.

4. Ammonium sulphamate, AR grade(Pharmaceutical Sciences Ltd., Part.)

5. Sodium hydroxide , AR grade (Merck) Lot.271.7966.

6. Monobasic potassium phosphate (Potassium dihydrogen phosphate), GR grade (Merck) Lot.313 A751273.

7. Disodium hydrogenphosphate 2-hydrate,GR grade (Merck) 423 K4194680.

8. Glycin, AR grade (Riedel-De Haen AG Seelze-Hanover) Lot. 890258-33226.

9. Sodium chloride, Extra pure (Vidhyasom Co., Ltd.) Lot.00630.

10. Trichloroacetic acid, AR grade (Merck) Lot.637 K2967707, 520 K602707.

11. Phenol, AR grade (Merck) Lot. 614 K1844406.

12. Ammonia solution 25 %, AR grade (Merck) Lot. 514 K410332.

C. Apparatus

1. Analytical balance (August Sauter KG D-7470, West Germany).

2. Spectrophotometer(Spectronic 2000, Bausch & Lomb, N.Y., USA)

3. Sartorius Absorption Simulator S.M. 16750 (Sartorius GmbH, West Germany)

4. Vortex mixer (Vortex-Genie, Scientific Industries Inc., Bohemia, N.Y. , USA)

5. Digital Computer (IBM Compatible 16 Bit, Micro Source)

Methods

A. In Vitro Study

1. Assay for content of active ingredient

One brand of paracetamol elixir and four brands of paracetamol suspensions were assayed using the official methods of B.P. 1980 and U.S.P. XXI, as follows.

1.1 Paracetamol elixir (11)

Paracetamol elixir, 1.5 g, was added with 100 ml of water and 20 ml 0.1 M sodium hydroxide before being diluted to 200 ml with water. Aliquot 5 ml of the solution was added with 9.5 ml of 0.1 M sodium hydroxide and then the mixture was diluted to 100 ml with water. The final solution was measured for the absorbance at the wavelength of maximum absorbance at 275 nm. The standard solution with known concentration was also assayed following the same method, and the amount of paracetamol, weight by volume, was calculated using the following equation :

$$Cu = Cs.\frac{Au}{As} \qquad \text{Eq...1}$$

where Cu was the concentration of the paracetamol elixir, mcg/ml, Cs was known concentration of the standard

solution, mcg/ml, Au was the absorbance of the paracetamol elixir and As was the absorbance of the standard solution.

1.2 Paracetamol Suspensions (9)

Standard preparation :- A solution having a known concentration of about 100 mcg/ml of paracetamol working standard was prepared.

Assay preparation :- An accurately measured volume of the suspension, equivalent about to 100 mg of paracetamol, was transferred to a 100 ml volumetric flask before being added with 60 ml of water. After being shaken for 30 minutes, the solution obtained was diluted to volume with water. Again, the aliquot 10 ml from this solution was transferred to 100 ml volumetric flask and was diluted to volume with water.

The standard preparation, the assay preparation and water which was used as a blank, were transferred to separate 50-ml volumetric flasks. Each solution was added with 2 ml of 6 N hydrochloric acid and 5 ml of sodium nitrite solution (1 in 10).After being allowed to stand for 15 minutes, each mixture was added with 5 ml of ammonium sulphamate solution (15 in 100) and was diluted with water to volume.The absorbance from the standard preparation and the assay preparation in 1-cm cells relative to blank at the wavelength of maximum absorbance, 430 nm were determined and the quantity of paracetamol in

the suspension, weight by volume, was calculated using the following equation :

$$Cu = Cs.\frac{Au}{As} \qquad \text{Eq...1}$$

where Cu was the concentration of the assay preparation, mcg/ml, Cs was known concentration of the standard preparation, mcg/ml, Au was the absorbance of the assay preparation and As was the absorbance of the standard preparation.

2. Study of the in vitro absorption (12,13)

2.1 The quantity of the sample

The paracetamol in suspension and elixir forms were evaluated. The amount of paracetamol, chosen to dissolve in phase I, was 60 mg (Appendices B and C).

2.2 The aqueous phases

Type	Phase I	Phase II
Initial volume	100 ml	100 ml
pH(±0.1) artificial gastric fluid	pH 3.0	pH 7.5
artificial intestinal fluid	pH 6.0	pH 7.8
Temperature	39±1 °C	39±1 °C

2.3 The lipid barriers

Two different lipid barriers were available for the Absorption Simulator, the artificial gastric barrier (SM 15701) and the artificial intestinal barrier (SM 15702). Both barriers were used once and were prepared shortly before the start of the experiment. The effective area of a barrier was 80 cm^2. The pores of a barrier were filled with a liquid lipid-phase, mixture of the following components :

Type	components (unit by weight)		
	N*	S_1^*	S_2^*
Lipid mixture for SM 15701	4.20	0.10	-
Lipid mixture for SM 15702	0.90	-	4.00

* The lipid components for artificial membranes.

The barrier was prepared by warming 4 to 5 g of the lipid mixture. A membrane filter was weighed and placed in the lipid mixture. After it was wetted as seen by complete transparency, the membrane filter was pressed between two pieces of blotting paper and was reweighed. The barriers should have a weight increasing of 85 to 105% for SM 15701, and 90 to 110% for SM 15702.

2.4 Sample collection

The samples were taken from each container at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 hours after experiment was begun. Each 3 ml sample was collected using

the taps on the distribution caps of the model. Every experiment was repeated triplicately.

2.5 Determination of paracetamol in aqueous phase I and phase II

The concentration of paracetamol in aqueous phase I and II was determined in a UV spectrophotometer at 245 nm using a standard curve.

Standard curve :- Standard solutions of paracetamol with concentrations of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 mcg/ml in aqueous phase I were determined in a UV spectrophotometer at 245 nm. Absorbances obtained versus known concentrations were fitted to a straight line using linear regression, (Appendix D).

2.6 Statistical evaluation of the in vitro absorption results

The diffusion rate constant, Kd and the absorption rate constant, Ki, of paracetamol elixir and suspensions were compared using a one-way analysis of variance and t-test through a computerized statistical program ABSTAT. Also the correlation of the in vitro absorption rate constant, Ki and the in vivo absorption rate constant, Ka, was evaluated.

B. In Vivo Study

1. Subjects

Eight healthy volunteers, 4 females and 4 males, aging range from 20-30 years and weighing between 45-65 kg were participated in this study, (Appendix E). They were taking not any medications, and having no history of gastro-intestinal, liver and renal diseases.

2. Dose and Drug Administration

The subjects received a 25-ml, equivalent to 600-mg paracetamol, as an oral single dose of each test products. Each dose was prepared by accurately pipetting either the elixir or the suspensions to a measuring glass and administering from it. The quantitative dosing was ensured by repeated washing of the measuring glasses with water to complete intake of 200 ml. The doses were given in the morning after overnight fast ; no food was permitted until two hours postdose.

3. Experimental design

The study was conducted in a crossover experiment. The dose was administered one week apart as shown in Table 1.

Table 1 Dosing Schedule

Subject No.	Week				
	1	2	3	4	5
1	E[a]	S1	S2	S3	S4
2	S1[b]	S2[b]	S3[b]	S4[b]	E
3	S2	S3	S4	E	S1
4	S3	S4	E	S1	S2
5	S4	E	S1	S2	S3
6	E	S1	S2	S3	S4
7	S1	S2	S3	S4	E
8	S2	S3	S4	E	S1

[a] E represents the brand name of paracetamol elixir.

[b] S1, S2, S3 and S4 represent the brand name of paracetamol suspensions.

4. Sample collection

Urine samples for paracetamol analysis were collected quantitatively prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24 and 32 hours after drug administration. Subjects were carefully instructed to deliver a complete urine specimen (i.e., completely emptying the bladder) (14). Aliquots of 15 ml from urine samples were stored at 2-8 ℃ until subsequent assay.

5. Determination of paracetamol in urine

5.1 Urine samples

Paracetamol and its metabolites in each urine sample was triplicately assayed using modified colorimetric method of Novotny and Elser(15). The procedure was developed as follows.

A 1.0 ml of appropriately dilute urine sample was transferred into 16x100 mm tubes. A 2.0 ml of the 2% trichloracetic acid reagent was then added and mixed using vortex-mixer for 5 seconds.A 1.0 ml of the mixture was sampled and transferred into a 16x150 mm tube. A 0.4 ml of 7 M hydrochloric acid was added and placed in a boiling water bath for 35 minutes.The tubes were removed from the water bath and the solutions were allowed to cool to room temperature. A 10.0 ml of freshly prepared 2 % phenol/4M ammonium hydroxide reagent, 7/3 by volume, was added to each tube and mixed. Finally, the solution was allowed to stand for 40-60 minutes and measured for the absorbance at 630 nm after having adjusted the absorbance of a urine blank to zero.

5.2 Standard curve

Standard solutions of paracetamol with concentrations of 20,40,60,80,100,120,140,160,180 and 200 mcg/ml in pooled urine were prepared and assayed following the same method as described earlier. Absorbances obtained

versus known concentrations were fitted to a straight line using linear regression (Appendix D).

6. Statistical evaluation of bioavailability results

The relative bioavailability was calculated using the following equation (16) :-

$$F_{rel} = \frac{[Du]_{\infty\,suspension}}{[Du]_{\infty\,elixir}} \times \frac{Dose_{elixir}}{Dose_{suspension}} \times 100 \qquad Eq...2$$

where, $[Du]_{\infty}$ was the maximum cumulative amount of paracetamol excreted into the urine.

The comparative bioavailability of paracetamol suspensions with paracetamol elixir and among paracetamol suspensions themselves were evaluated using the following parameters: (a) the maximum cumulative amount of drug excreted into the urine, $[Du]_{\infty}$, (b) the rate of drug excretion , dDu/dt, (c) the time for maximum urinary excretion, t_{∞} and (d) the absorption rate constant, Ka. A one-way analysis of variance and t-test were used through a computerized statistical program ABSTAT for data analysis. Moreover, other pharmacokinetic parameters were calculated (e.g., elimination rate constant, K ; half-life, $t_{1/2}$ and lag time, Tlag).